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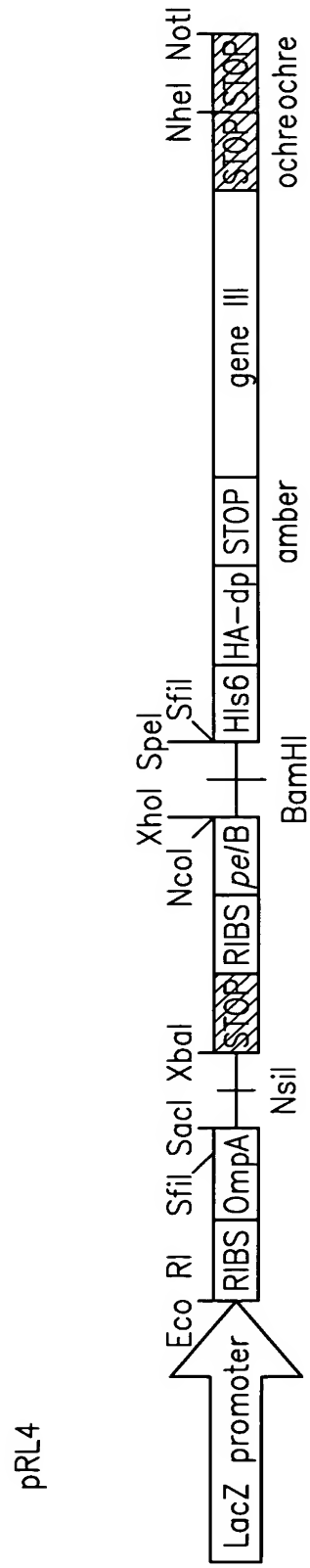


FIG. 1

Human Antibody sequence (TT sequence) (SEQ. ID NO: 54)
Heavy Chain: cloning sites Xho I and Spe I are underlined

```
1                                                   11
gag gtg cag ctg CTC GAG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG TCC TCG GTG AAG
glu val gln leu leu glu gln ser gly ala glu val lys lys pro gly ser ser val lys
21                                                  31
GTC TCC TGC AGG GCT TCT GGA GGC ACC TTC AAC AAT TAT GCC ATC AGC TGG GTG CGA CAG
val ser cys arg ala ser gly gly thr phe asn asn tyr ala ile ser trp val arg gln
41                                                  51
GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA GGG ATC TTC CCT TTC CGT AAT ACA GCA AAG
ala pro gly gln gly leu glu trp met gly gly ile phe pro phe arg asn thr ala lys
61                                                  71
TAC GCA CAA CAC TTC CAG GGC AGA GTC ACC ATT ACC GCG GAC GAA TCC ACG GGC ACA GCC
tyr ala gln his phe gln gly arg val thr ile thr ala asp glu ser thr gly thr ala
81                                                  91
TAC ATG GAG CTG AGC AGC CTG AGA TCT GAG GAC ACG GCC ATA TAT TAT TGT GCG AGA GGG
tyr met glu leu ser ser leu arg ser glu asp thr ala ile tyr tyr cys ala arg gly
101                                                 111
GAT ACG ATT TTT GGA GTG ACC ATG GGA TAC TAC GCT ATG GAC GTC TGG GGC CAA GGG ACC
asp thr ile phe gly val thr met gly tyr tyr ala met asp val trp gly gln gly thr
121                                                 131
ACG GTC ACC GTC TCC GCA GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC cTG GCA CCC TCC
thr val thr val ser ala ala ser thr lys gly pro ser val phe pro leu ala pro ser
141                                                 151
TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC
ser lys ser thr ser gly gly thr ala ala leu gly cys leu val lys asp tyr phe pro
161                                                 171
GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG
glu pro val thr val ser trp asn ser gly ala leu thr ser gly val his thr phe pro
181                                                 191
GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC
ala val leu gln ser ser gly leu tyr ser leu ser ser val val thr val pro ser ser
201                                                 211
AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG
ser leu gly thr gln thr tyr ile cys asn val asn his lys pro ser asn thr lys val
221                                                 231
GAC AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA act agt
asp lys lys val glu pro lys ser cys asp lys thr ser
```

FIG. 2A

Human Antibody sequence (TT sequence) (SEQ. ID NO: 55)
Light Chain: cloning sites Sac I and Xba I are underlined

```
1                                                   11
gag ctc acg cag tct cca ggc acc ctg tct ttg tct ccA ggg gaa aga gcc acc ctc tcc
glu leu thr gln ser pro gly thr leu ser leu ser pro gly glu arg ala thr leu ser
21                                                  31
tgc agg gcc agt cac agt gtt agc agg gcc tac tta gcc tgg tac cag cag aaa cct ggc
cys arg ala ser his ser val ser arg ala tyr leu ala trp tyr gln gln lys pro gly
41                                                  51
cag gct ccc agg ctc ctc atc tat ggt aca tcc agc agg gcc act ggc atc cca gac agg
gln ala pro arg leu leu ile tyr gly thr ser ser arg ala thr gly ile pro asp arg
61                                                  71
ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc agc aga ctg gag cct gaa
phe ser gly ser gly ser gly thr asp phe thr leu thr ile ser arg leu glu pro glu
81                                                  91
gat ttt gca gtg tac tac tgt cag cag tat ggt ggc tca ccg tgg ttc ggc caa ggg acC
asp phe ala val tyr tyr cys gln gln tyr gly gly ser pro trp phe gly gln gly thr
101                                                 111
AAG GTG GAA CTC AAA CGA ACT GTG GCT GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT
lys val glu leu lys arg thr val ala ala pro ser val phe ile phe pro pro ser asp
121                                                 131
GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA
glu gln leu lys ser gly thr ala ser val val cys leu leu asn asn phe tyr pro arg
141                                                 151
GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT
glu ala lys val gln trp lys val asp asn ala leu gln ser gly asn ser gln glu ser
161                                                 171
GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC
val thr glu gln asp ser lys asp ser thr tyr ser leu ser ser thr leu thr leu ser
181                                                 191
AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC
lys ala asp tyr glu lys his lys val tyr ala cys glu val thr his gln gly leu ser
201                                                 211
TTG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT TAG Ttc tag a
leu pro val thr lys ser phe asn arg gly glu cys AMB
```

FIG. 2B

Method of grafting peptide into antibody with random sequences surrounding peptide sequence

	FR3							TPO Mimetic Peptide							
(SEQ ID NO. 56)	Y	Y	C	A	R	X	X	I	E	G	P	T	L	R	Q
(SEQ ID NO. 57)	TATTAT-	TGT-	GCG-	AGA-	NNR-	NNR-		ATT-	GAA-	GGG-	CCG-	ACG-	CTG-	CGG-	CAA-

								FR4				
W	L	A	A	R	A	X	X	W	G	Q	G	T
TGG-	CTG-	GCG-	GCG-	CGC-	GCG-	NNY-	NNY-	TGG-	GGC-	CAA-	GGG-	ACC-

The TPO mimetic peptide was grafted into the heavy chain CDR3 region of the tetanus toxoid antibody. The peptide was flanked on either side by two random amino acids, shown as "X"s in the figure.

FIG. 3

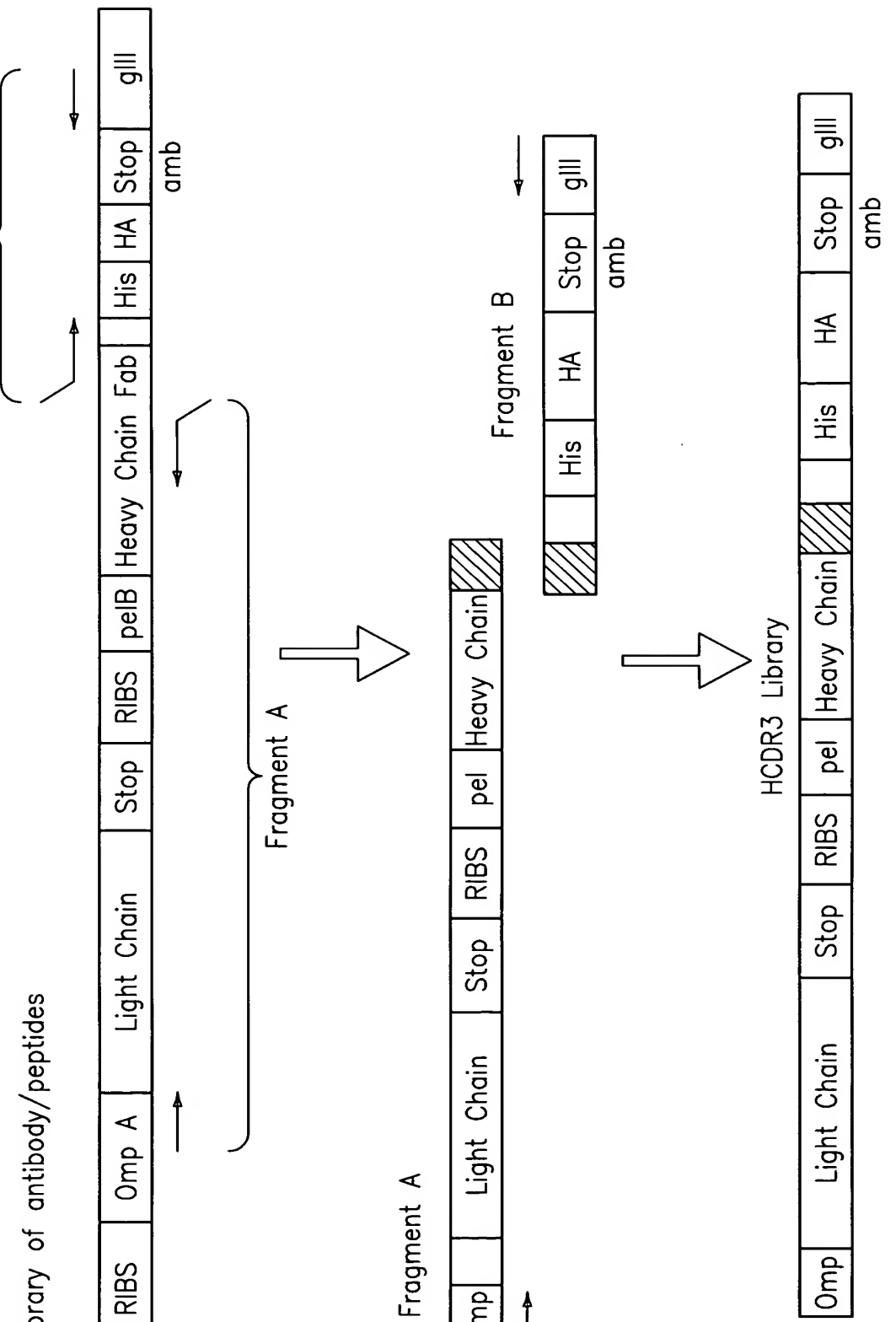

Library of antibody/peptides
RIBS
Omp A
Light Chain
Stop
RIBS
pelB
Heavy Chain Fab
His
HA
Stop
gIII
amb
Fragment B
Fragment A
Fragment A
Omp
Light Chain
Stop
RIBS
pel
Heavy Chain
Fragment B
His
HA
Stop
gIII
amb
HCDR3 Library
Omp
Light Chain
Stop
RIBS
pel
Heavy Chain
His
HA
Stop
gIII
amb

FIG. 4

CLONE	AMINO ACID SEQUENCE	SEQ ID NO.
X1a	Pro-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly	25
	CCG-CCC-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-GGA-GGC	26
X1a-11	Gly-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly	27
	GGG-GGT-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-GGC-GGA	28
X1a-13	Gly-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly	29
	GGC-GGT-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-GGA-GGC	30
X1c	Trp-Leu-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val	31
	TGG-CTG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GTC	32
X2c	Mat-Ile-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Val-Gly	33
	ATG-ATA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-GTT-GGC	34
X3a	Val-Val-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val	35
	GTG-GTA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GTT	36
X3b	Gly-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Asp	37
	GGG-CCG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCC-GAT	38
X4b	Leu-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val	39
	TTG-CCA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GTT	40
X4c	Ser-Leu-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Ile	41
	TCA-CTG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCC-ATC	42
X5a	Thr-Met-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val	43
	ACA-ATG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCC-GTT	44
X5c	Thr-Thr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val	45
	ACG-ACA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GTC	46
X7a	Thr-Arg-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Cys-Ser	47
	ACA-CGG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-TGC-AGC	48
X7b	no peptide deletion mutant	
X7c	Gln-Thr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Asp	49
	CAG-ACA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-CAC	50

FIG. 5

pRL8

(SEQ ID NO: 60)

GGGAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAA
ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATC
AAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGT
CCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATC
AGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTC
GAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGA
GCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGC
GAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGT
AACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGC
ACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACA
TTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAAT
ATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCC
TTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAA
GTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGG
ATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCA
ATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGA
CGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTG
GTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAA
GAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTT
ACTTCTGACAACGATGGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAAC
ATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAG
CCATACCAAACGACGAGCTGTACACCACGATGCCTGTAGCAATGGCAACAAC
GTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAAT
TAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGC
CCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGT
CTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGT
AGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACA
GATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAA
GTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGG
ATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGA
GTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTT
GAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCG
CTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAA
GGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAG
CCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGC
TCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTA
CCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTG
AACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGA

FIG. 6A

ACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGG
AGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGC
ACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGT
TTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGG
AGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTG
CTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAA
CCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACC
GAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAA
CCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTT
TCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTC
ACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTG
TGGAATTGTGAGCGGATAACAATTGAATTCAGGAGGAATTTAAAATGAAAAA
GACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTTCGCTACCGTGGCCCAG
GCGGCCGAGCTCGGCCATGGCTGGTTGGGCAGCGAGTAATAACAATCCAGCG
GCTGCCGTAGGCAATAGGTATTTCATTATGACTGTCTCCTTGGCGACTAGCTA
GTTTAGAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCC
GCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGG
GGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGC
TTTCCAGTCGGGAAACCTGTCGTGTTACTAATGATGGTGATGGTGATGGCTAG
TTTTGTCACAAGATTTGGGCTCAACTTTCTTGTCCACCTTGGTGTTGCTGGGCT
TGTGATTCACGTTGCAGATGTAGGTCTGGGTGCCCAAGCTGCTGGAGGGCAC
GGTCACCACGCTGCTGAgGGAGTAGAGTCCTGAGGACTGTAGGACAGCCGGG
AAGGTGTGCACGCCGCTGGTCAgGGCGCCTGAgTTCCACGACACcGTCGCCGG
TTCgGGGAAGTAGTCCTTGACCAGGCAGCCCAGGGCCGCTGTGCCCCCAGAG
GTGCTCTTGGAGGAGGGTGCCAGGGGGAAGACCGATGGGCCCTTGGTGGAG
GCTGCGGAGACGGTGACCGTGGTACCAGCAGAAACCTGGCCAGGCTCCCAG
GCTCCTCATCTATGGTACATCCAGCAGGGCCACTGGCATCCCAGACAGGTTC
AGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC
CTGAAGATTTTGCAGTGTACTACTGTCAGCAGTATGGTGGCTCACCGTGGTTC
GGCCAAGGGACCAAGGTGGAACTCAAACGAACTGTGGCTGCACCATCTGTCT
TCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTG
TGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGG
ATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAG
CAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA
CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCacccatcagggcctgagttcgcccgtcac
aaagagcttcaacggaggagagtgttaatTCTAGATAATTAATTAGGAGGAATTTAAAATGAA
ATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAG
CCATGGCCGAGGTGCAGCTGCTCGAGATGAGCGATAAAATTATTCACCTGAC
TGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCCTCGTC
GATTTCTGGGCAGAGTGGTGCGGTCCGTGCAAAATGATCGCCCCGATTCTGG
ATGAAATCGCTGACGAATATCAGGGCAAACTGACCGTTGCAAAACTGAACAT
CGATCAAAACCCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACT
CTGCTGCTGTTCAAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTTG
TCTAAAGGTCAGTTGAAAGAGTTCCTCGACGCTAACCTGGCGTACCCGTACG
ACGTTCCGGACTACGGTTCTACTAGTccgaaaccgtctaccccaccgggctcttcctgcggtggccgc
atcgcccgtctggaggaaaaagtgaaaaccctgaaagctcagaactccgagctggcgtccactgccaacatgctgcgcgaac

FIG. 6B

aggtggcacagctgaaacagaaagttatgaaccatggcggttgtgctagtGGCCAGGCCGGCCAGCACCAT
CACCATCACCATGGCGCATACCCGTACGACGTTCCGGACTACGCTTCTTAGG
AGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGG
CGGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCAA
ACGCTAATAAGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTC
TGACGCTAAAGGCAAACTTGATTCTGTCGCTACTGATTACGGTGCTGCTATCG
ATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGT
GATTTTGCTGGCTCTAATTCCCAAATGGCTCAAGTCGGTGACGGTGATAATTC
ACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGA
ATGTCGCCCTTTTGTCTTTAGCGCTGGTAAACCATATGAATTTTCTATTGATTG
TGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCAC
CTTTATGTATGTATTTTCTACGTTTGCTAACATACTGCGTAATAAGGAGTCTTA
AGCTAGCTAATTAATTTAAGCGGCCGCAGATCT

FIG. 6C

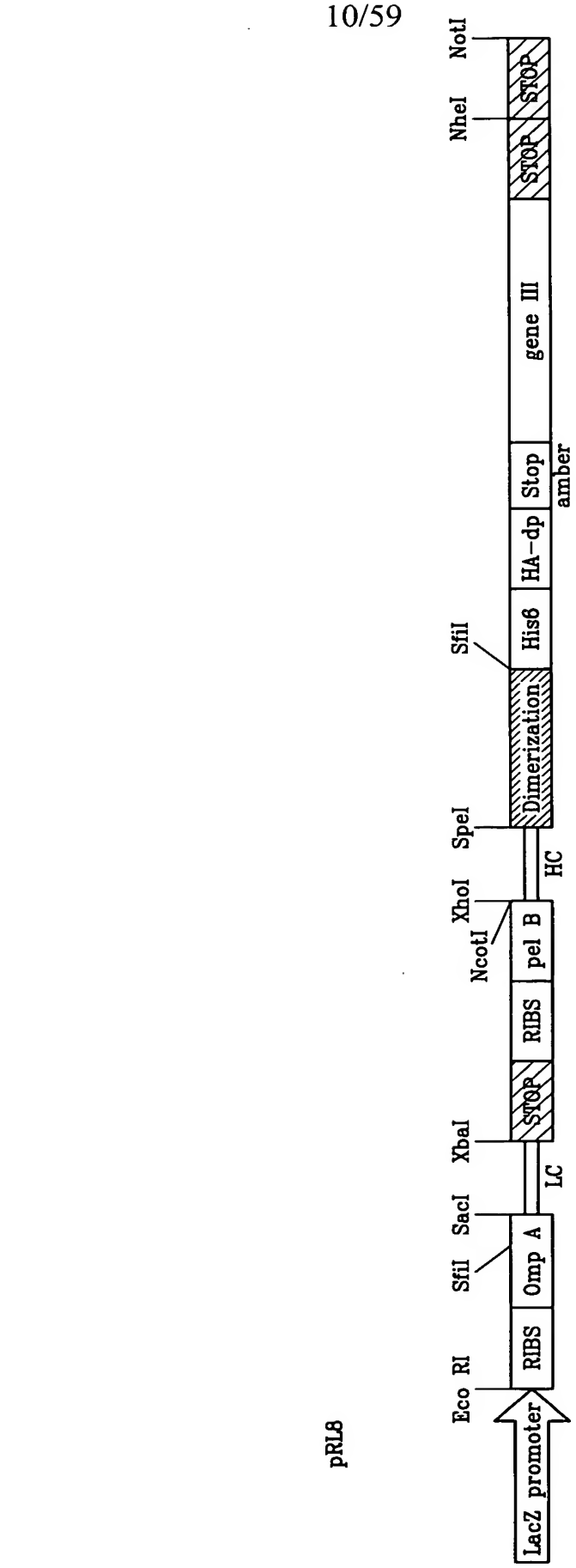

pRL8
LacZ promoter
Eco RI
RIBS
SfiI
Omp A
SacI
LC
XbaI
STOP
RIBS
pel B
NcoI
XhoI
HC
SpeI
Dimerization
SfiI
His6
HA-dp
Stop
amber
gene III
STOP
STOP
NheI
NotI

FIG. 7

Spe I
(SEQ ID NO: 52) ACTAGTCCGAAACCGTCTACCCCACCGGGCTCTTCCTGCGGTGGCCGCATCGCCCGTCTGGAGGAAAAAGTGAAAACCCTGAAAGCTCAGAACTCCGAGCTGGCGTCCACTGCCA
115
(SEQ ID NO: 53) P K P S T P P G S S C G G R I A R L E E K V K T L K A Q N S E L A S T A
hinge
jun dimerization domain
Sfi I
ACATGCTGCGCGAACAGGTGGCACAGCTGAAACAGAAAGTTATGAACCATGGCGGTTGTGCTAGTGGCCAGGCCGGCCAGCACCATCACCATCACCATGGCGCATACCCGTACGA
230
N M L R E Q V A Q L K Q K V M N H G G C A S G Q A G Q H H H H H H G A Y P Y D
jun dimerization domain
His 6 Tag
CGTTCCGGACTACGCTTCTTAGGAGGGTGGTGGCTCTGAG
270
V P D Y A S
HA tag and Amber stop

FIG. 8

TPO Positive Clones nnk nnk nnk nnk

1 2 IEGPTLRQWLAARA 3 4

	Sample	nnk 1	nnk 2	nnk 3	nnk 4	Amino Acids 1	Amino Acids 2	Amino Acids 3	Amino Acids 4
HC CDR3	X1c	tgg	ctg	cct	gtc	Trp	Leu	Pro	Val
	X3a	gtg	gta	cct	gtt	Val	Val	Pro	Val
	X3b	ggg	gta	ccc	gat	Gly	Pro	Pro	Asp
	X4b	ttg	cca	cct	gtt	Leu	Pro	Pro	Val
	X4c	tca	ctg	ccc	atc	Ser	Leu	Pro	Ile
	X5a	aca	atg	ccc	gtt	Thr	Met	Pro	Val
	X5c	acg	aca	cct	gtc	Trp	Leu	Pro	Val
	X7c	cag	aca	cct	cac	Gln	Thr	Pro	Asp
HC CDR2	24	ctt	tat	tct	aat	Leu	Tyr	Ser	Asn
	39	act	tac	ttg	cat	Thr	Tyr	Leu	His
	3	agg	atg	ctc	gag	Arg	Met	Leu	Glu
	7	aag	gaa	tct	aag	Lys	Glu	Ser	Lys
	8	gcg	cat	gtg	cag	Ala	His	Val	Gln
	10	cag	gag	att	agt	Gln	Glu	Ile	Ser
	11	cgg	aat	aat	ccg	Arg	Asn	Asn	Pro
	19	cag	cta	aat	tct	Gln	Leu	Asn	Ser
	25	agt	att	ttt	gtc	Ser	Ile	Phe	Val
	28	ggg	ccc	act	agt	Gly	Pro	Thr	Ser
LC CDR1	10	aag	ggt	gtt	agt	Lys	Gly	Val	Ser
	11	cat	ggg	gtg	gct	His	Gly	Val	Ala
	12a	cgt	acg	atg	gct	Arg	Thr	Met	Ala
	12b	cgt	ggt	gtt	aat	Arg	Gly	Val	Asn
	14	cgt	tcg	ctt	gcg	Arg	Ser	Leu	Ala
	16	cgg	ggt	gtt	gcg	Arg	Gly	Val	Ala
	18	agg	acg	gtg	tct	Arg	Thr	Val	Ser
	47	aag	ggg	gtg	gcg	Lys	Gly	Val	Ala
LC CDR2	1	aat	ccg	agg	ggt	Asn	Pro	Arg	Gly
	2	tcg	cct	cgg	agt	Ser	Pro	Arg	Ser
	3	tcg	cct	cgt	acg	Ser	Pro	Arg	Thr
	4	tcg	cct	tgg	cgt	Ser	Pro	Trp	Arg
	5	act	ccg	aat	tgg	Thr	Pro	Asn	Trp
	6	aat	cct	gcg	agg	Asn	Pro	Ala	Arg
	7	aat	ccg	tcg	ggg	Asn	Pro	Ser	Gly
	9	aat	cct	tat	tag	Asn	Pro	Tyr	Stop
	10	aat	ccg	cgg	tct	Asn	Pro	Arg	Ser
	11	aat	ccg	gat	gtg	Asn	Pro	Asp	Val
	12	tcg	ccg	tcg	cgg	Ser	Pro	Ser	Arg
	13	aat	cct	ctg	ttt	Asn	Pro	Leu	Phe
	14	aat	ctt	ggg	tat	Asn	Pro	Gly	Tyr
	15	aat	cct	att	agt	Asn	Pro	Ile	Ser
	16	aat	cct	cag	cgg	Asn	Pro	Gln	Arg
	18	aat	ccg	cgg	acg	Asn	Pro	Arg	Thr
	19	aat	ccg	cgt	ggg	Asn	Pro	Arg	Gly
	20	cat	ttg	aga	ctg	His	Leu	Arg	Leu
	21	aag	tag	att	tat	Lys	Stop	Ile	Tyr
	23	aat	cct	ggt	aag	Asn	Pro	Gly	Lys
	24	aat	cct	cgt	ggg	Asn	Pro	Arg	Gly
	26	aat	cct	aat	gtg	Asn	Pro	Asn	Val
	27	tct	ccg	cgg	gtt	Ser	Pro	Arg	Val
	29	acg	cct	cgg	ggt	Thr	Pro	Arg	Gly
	30	?ct	tag	tgg	tgg	Pro	Stop	Trp	Trp

FIG. 9

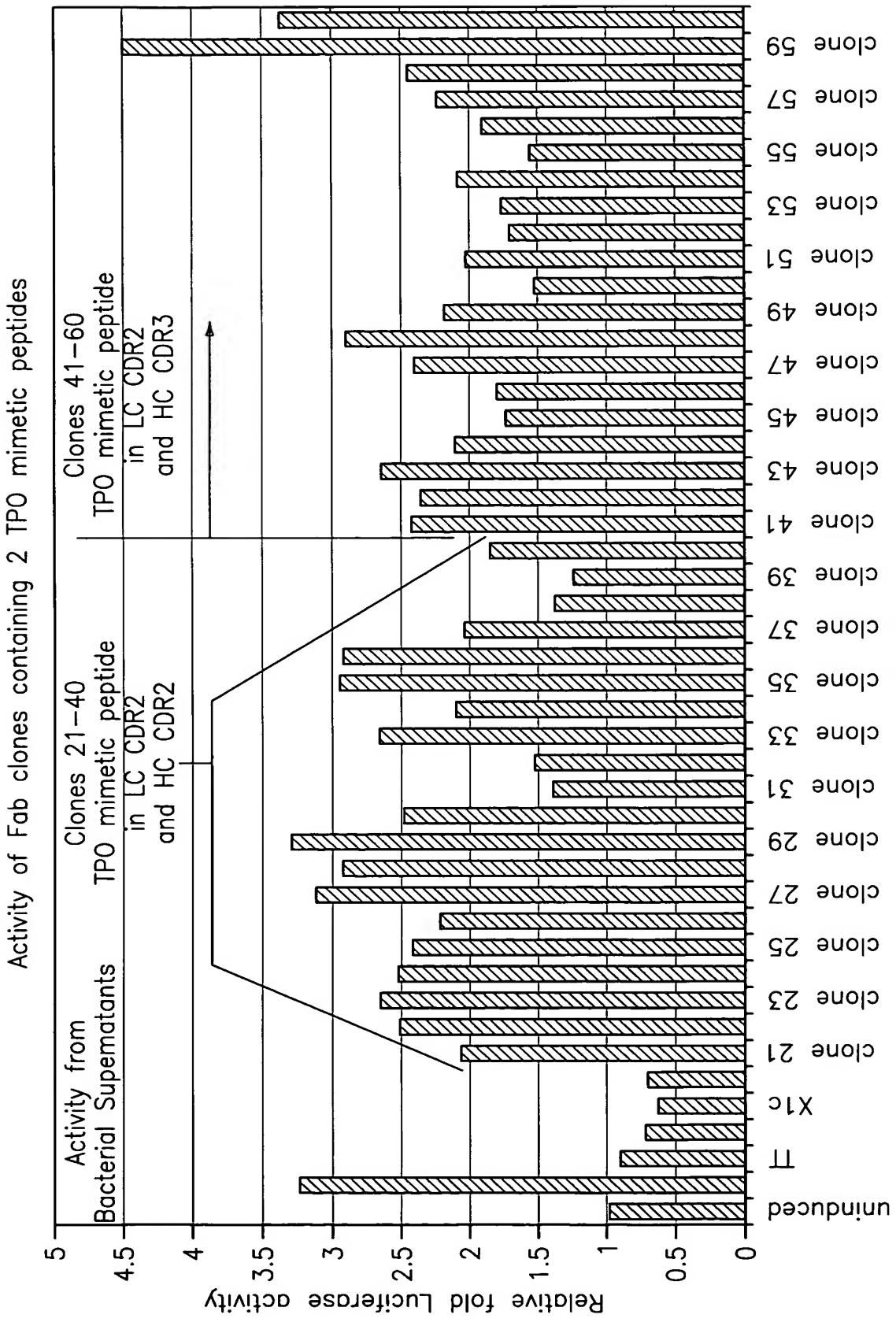

Activity of Fab clones containing 2 TPO mimetic peptides
Activity from Bacterial Supematants
Clones 21–40 TPO mimetic peptide in LC CDR2 and HC CDR2
Clones 41–60 TPO mimetic peptide in LC CDR2 and HC CDR3
Relative fold Luciferase activity
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
uninduced
TT
X1c
clone 21
clone 23
clone 25
clone 27
clone 29
clone 31
clone 33
clone 35
clone 37
clone 39
clone 41
clone 43
clone 45
clone 47
clone 49
clone 51
clone 53
clone 55
clone 57
clone 59
Test Samples

FIG. 10

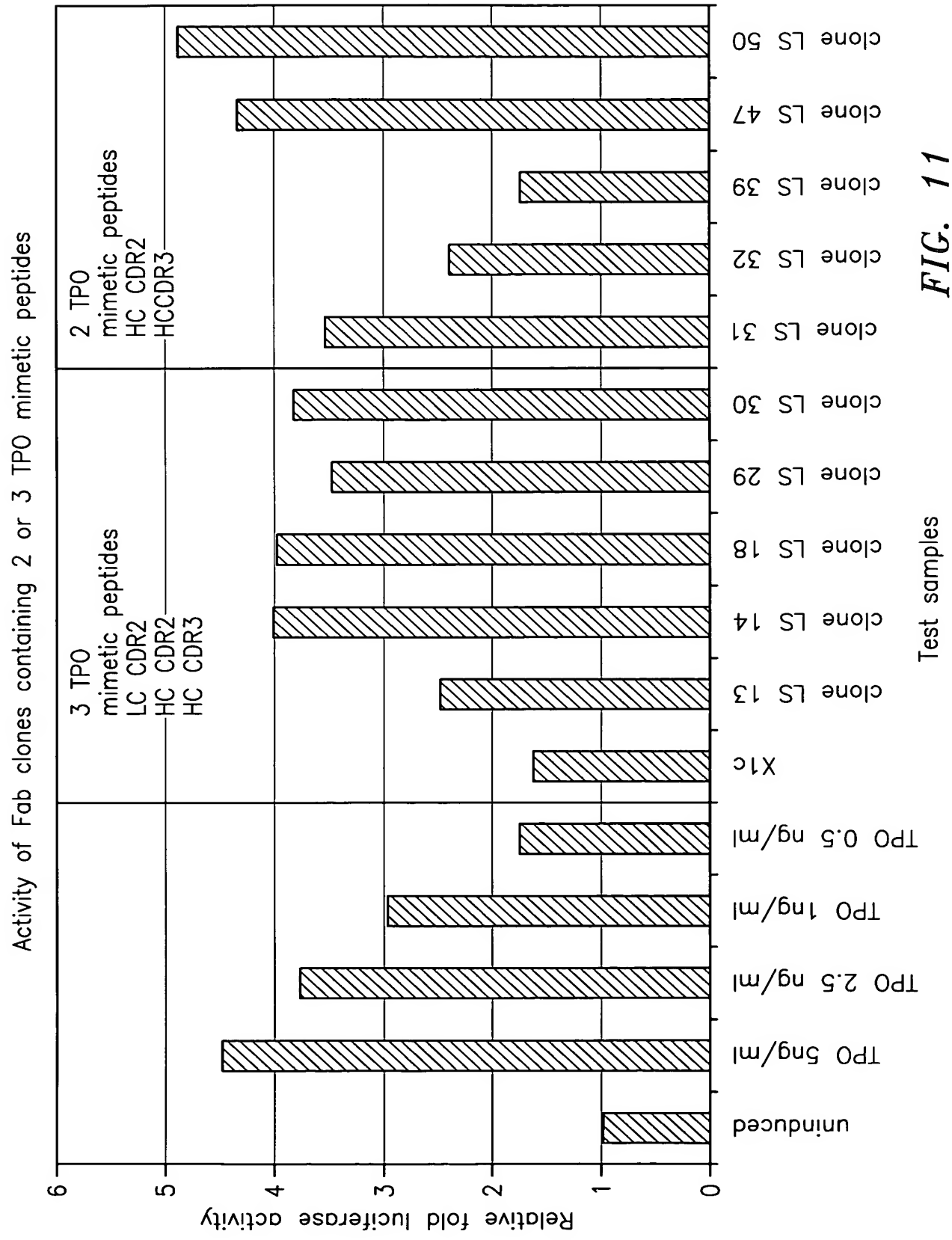

Activity of Fab clones containing 2 or 3 TPO mimetic peptides
Relative fold luciferase activity
0
1
2
3
4
5
6
3 TPO mimetic peptides LC CDR2 HC CDR2 HC CDR3
2 TPO mimetic peptides HC CDR2 HCCDR3
uninduced
TPO 5ng/ml
TPO 2.5 ng/ml
TPO 1ng/ml
TPO 0.5 ng/ml
X1c
clone LS 13
clone LS 14
clone LS 18
clone LS 29
clone LS 30
clone LS 31
clone LS 32
clone LS 39
clone LS 47
clone LS 50
Test samples

FIG. 11

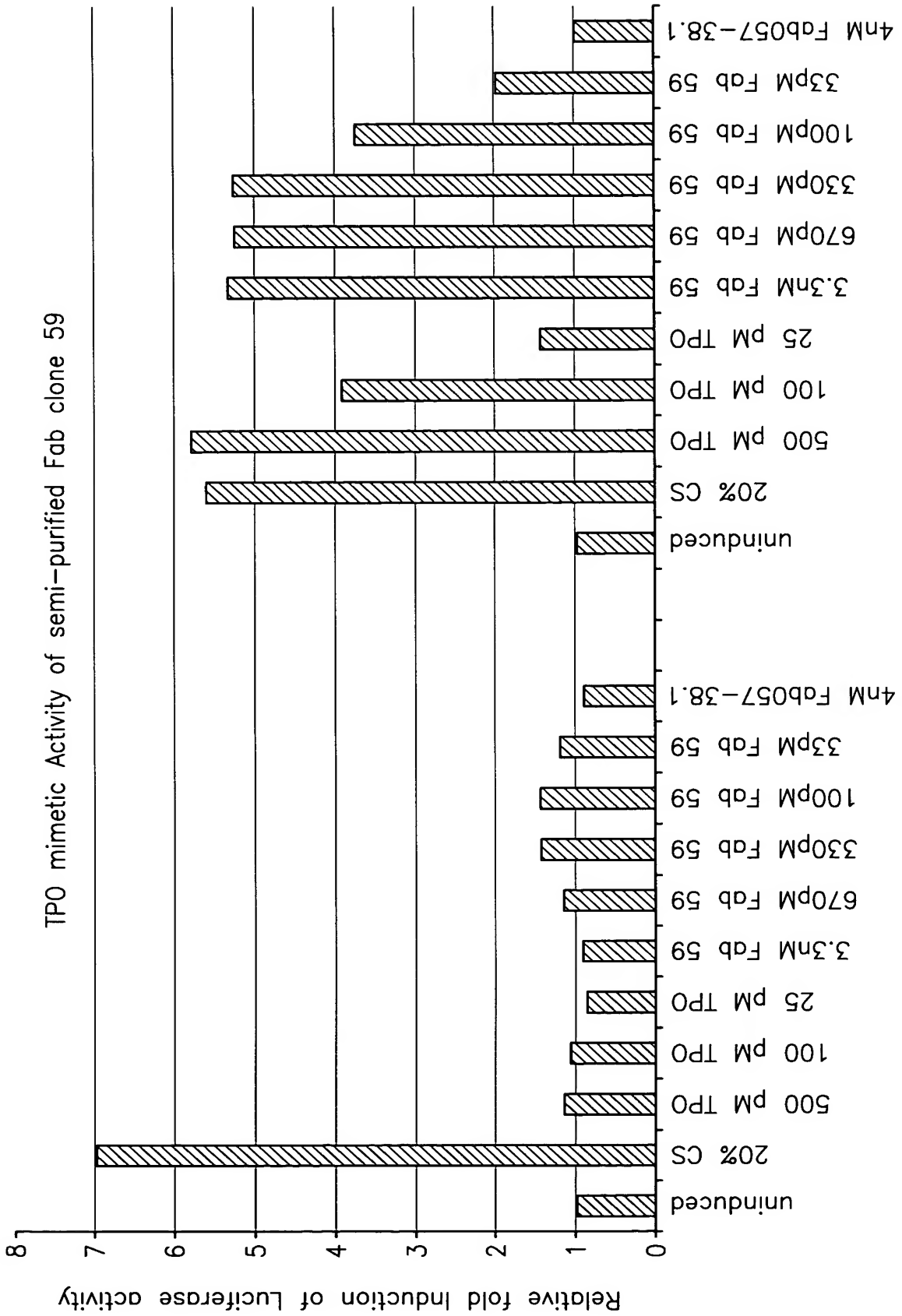

TPO mimetic Activity of semi-purified Fab clone 59
Relative fold Induction of Luciferase activity
0
1
2
3
4
5
6
7
8
uninduced
20% CS
500 pM TPO
100 pM TPO
25 pM TPO
3.3nM Fab 59
670pM Fab 59
330pM Fab 59
100pM Fab 59
33pM Fab 59
4nM Fab057-38.1
uninduced
20% CS
500 pM TPO
100 pM TPO
25 pM TPO
3.3nM Fab 59
670pM Fab 59
330pM Fab 59
100pM Fab 59
33pM Fab 59
4nM Fab057-38.1
Stimulation Conditions

FIG. 12

(SEQ ID NO: 67)

5G1.1-TPO Heavy Chain (Bold denotes TPO mimetic) Amino acid sequence:

*MKWSWVILFLLSVTAGVHS*QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQW
VRQAPGQGLEWMGEILPGSGSTEYTENFKDRVTMTRDTSTSTVYMELSSLRSED
TAVYYCARLPIEGPTLRQWLAARAPVWGQGTLVTVSSASTKGPSVFPLAPCSR
STSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRL
TVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK.

(SEQ ID NO: 68)

5G1.1-TPO Heavy Chain (Bold denotes TPO mimetic) Nucleic acid sequence:

ATGAAGTGGAGCTGGGTTATTCTCTTCCTCCTGTCAGTAACTGCCGGCGTCCA
CTCCCAAGTCCAACTGGTGCAATCCGGCGCCGAGGTCAAGAAGCCAGGGGCC
TCAGTCAAAGTGTCCTGTAAAGCTAGCGGCTATATTTTTTCTAATTATTGGAT
TCAATGGGTGCGTCAGGCCCCCGGGCAGGGCCTGGAATGGATGGGTGAGATC
TTACCGGGCTCTGGTAGCACCGAATATACCGAAAATTTTAAAGACCGTGTTA
CTATGACGCGTGACACTTCGACTAGTACAGTATACATGGAGCTCTCCAGCCTG
CGATCGGAGGACACGGCCGTCTATTATTGCGCGCGTTTGCCAATTGAAGGG
CCGACGCTGCGGCAATGGCTGGCGGCGCGCGCCTGTTTGGGGTCAAG
GAACCCTGGTCACTGTCTCGAGCGCCTCCACCAAGGGCCCATCCGTCTTCCCC
CTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGC
CCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCT
ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGAC
CTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAC
AGTTGAGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCAGCACCACCTGTG
GCAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGAT
CTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGAC
CCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCA
AGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCG
TCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAA
GGTCTCCAACAAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCC
AAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAG
GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACC
CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACT
ACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC
AGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCT
CCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCT
GTCTCTGGGTAAATGA

FIG. 13A

(SEQ ID NO: 69)

5G1.1 Light Chain Amino Acid Sequence

*MDMRVPAQLLGLLLLWLRGARC*DIQMTQSPSSLSASVGDRVTITCGASENIYGALN
WYQQKPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
NVLNTPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC.

(SEQ ID NO: 70)

5G1.1 Light Chain Nucleic Acid Sequence

ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTACTCTGGCTCCG
AGGTGCCAGATGTGATATCCAGATGACCCAGTCCCCGTCCTCCCTGTCCGCCT
CTGTGGGCGATAGGGTCACCATCACCTGCGGCGCCAGCGAAAACATCTATGG
CGCGCTGAACTGGTATCAACAGAAACCCGGGAAAGCTCCGAAGCTTCTGATT
TACGGTGCGACGAACCTGGCAGATGGAGTCCCTTCTCGCTTCTCTGGATCCGG
CTCCGGAACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCTGAAGACTTC
GCTACGTATTACTGTCAGAACGTTTTAAATACTCCGTTGACTTTCGGACAGGG
TACCAAGGTGGAAATAAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCC
CGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTG
AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACA
GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGA
AACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGT
CACAAAGAGCTTCAACAGGGGAGAGTGTTAG

Note: Italics denotes leader sequence

FIG. 13B

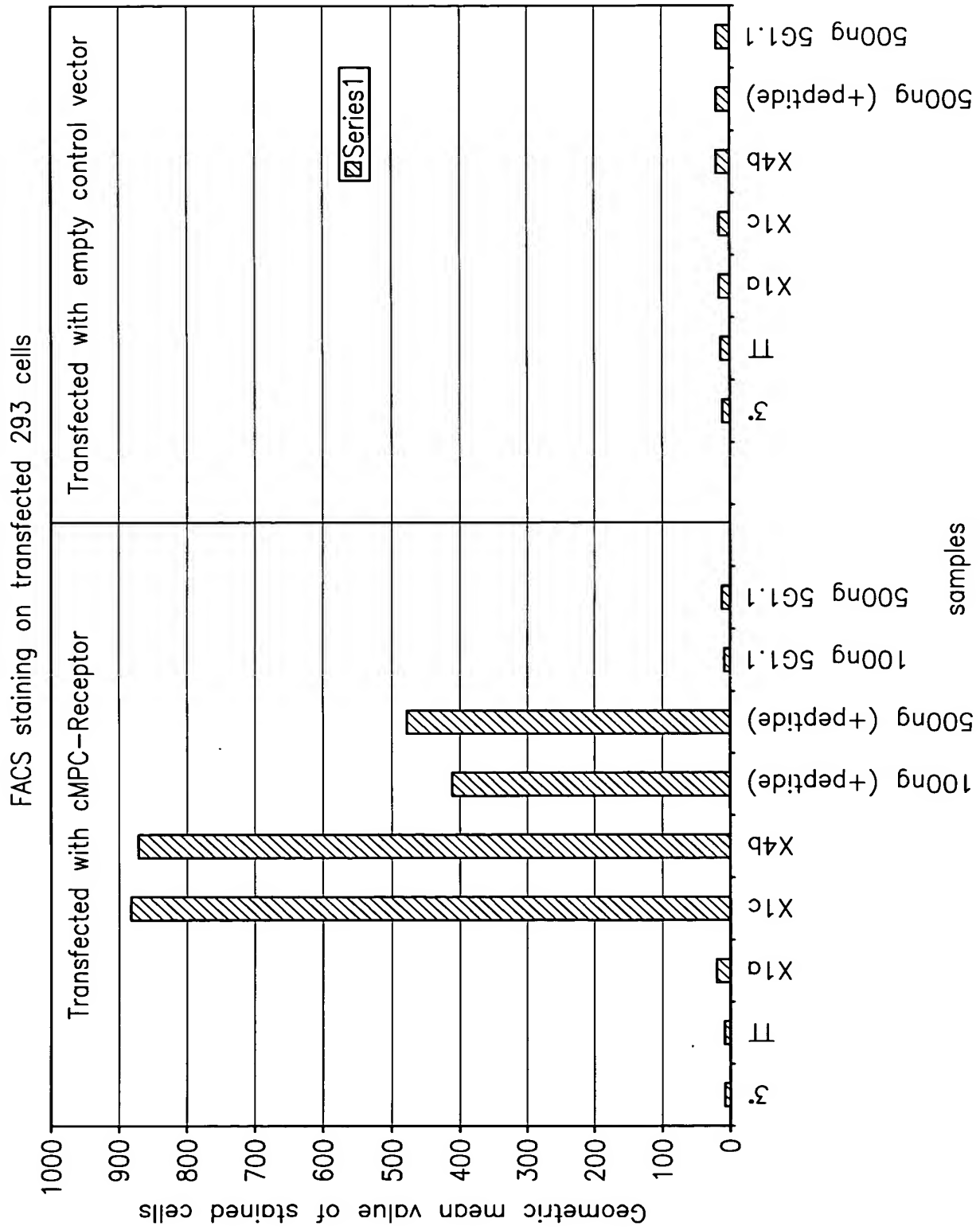

FACS staining on transfected 293 cells
Transfected with cMPC-Receptor
Transfected with empty control vector
Series1
Geometric mean value of stained cells
1000
900
800
700
600
500
400
300
200
100
0
3'
TT
X1a
X1c
X4b
100ng (+peptide)
500ng (+peptide)
100ng SG1.1
500ng SG1.1
samples

FIG. 14

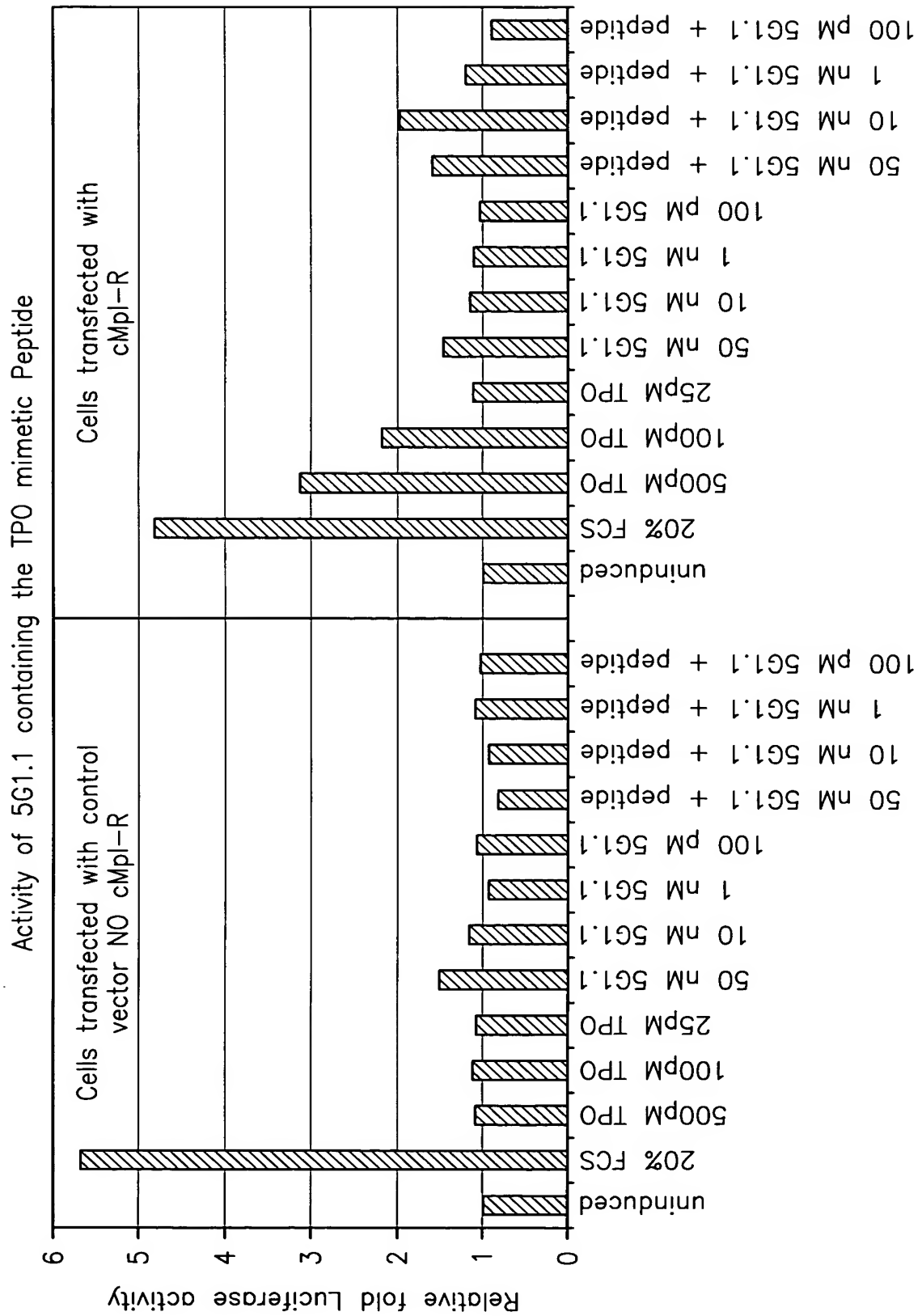

Activity of 5G1.1 containing the TPO mimetic Peptide
Relative fold Luciferase activity
0
1
2
3
4
5
6
Cells transfected with control vector NO cMpl-R
Cells transfected with cMpl-R
uninduced
20% FCS
500pM TPO
100pM TPO
25pM TPO
50 nM 5G1.1
10 nM 5G1.1
1 nM 5G1.1
100 pM 5G1.1
50 nM 5G1.1 + peptide
10 nM 5G1.1 + peptide
1 nM 5G1.1 + peptide
100 pM 5G1.1 + peptide
uninduced
20% FCS
500pM TPO
100pM TPO
25pM TPO
50 nM 5G1.1
10 nM 5G1.1
1 nM 5G1.1
100 pM 5G1.1
50 nM 5G1.1 + peptide
10 nM 5G1.1 + peptide
1 nM 5G1.1 + peptide
100 pM 5G1.1 + peptide
Test Samples

FIG. 15

VARIABLE REGION OF 4-29 LIGHT CHAIN

(SEQ ID NO: 116)

```
                                                
```

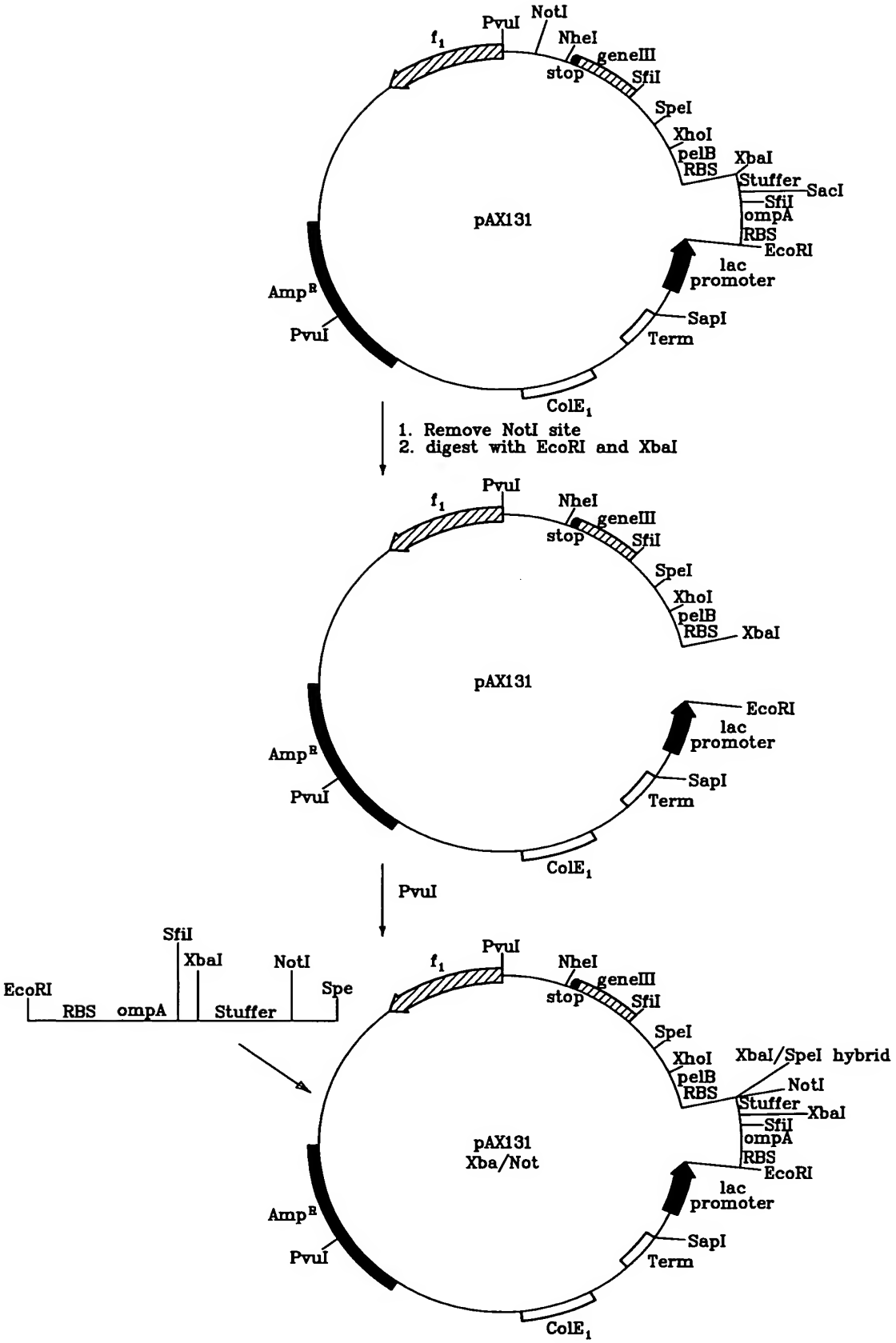

PvuI
NotI
f1
NheI
geneIII
stop
SfiI
SpeI
XhoI
pelB
RBS
XbaI
Stuffer
SacI
SfiI
ompA
RBS
EcoRI
pAX131
lac
promoter
AmpR
PvuI
SapI
Term
ColE1
1. Remove NotI site
2. digest with EcoRI and XbaI
PvuI
f1
NheI
geneIII
stop
SfiI
SpeI
XhoI
pelB
RBS
XbaI
pAX131
EcoRI
lac
promoter
AmpR
PvuI
SapI
Term
ColE1
PvuI
SfiI
XbaI
NotI
EcoRI
Spe
RBS
ompA
Stuffer
PvuI
f1
NheI
geneIII
stop
SfiI
SpeI
XhoI
XbaI/SpeI hybrid
pelB
RBS
NotI
Stuffer
XbaI
SfiI
ompA
RBS
EcoRI
pAX131
Xba/Not
lac
promoter
AmpR
PvuI
SapI
Term
ColE1

FIG. 17

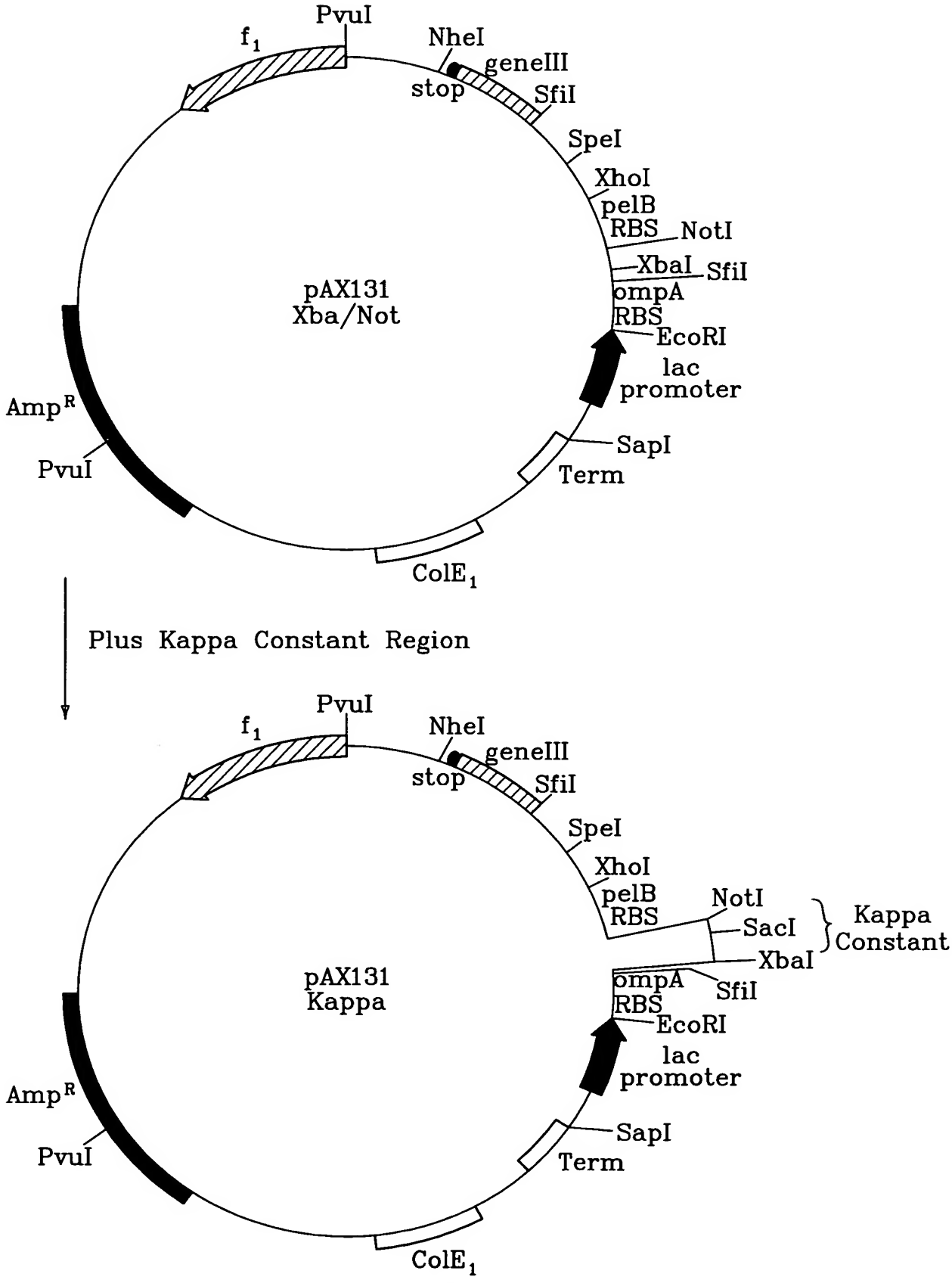

PvuI
f1
NheI
geneIII
stop
SfiI
SpeI
XhoI
pelB
RBS
NotI
XbaI
SfiI
ompA
RBS
EcoRI
lac
promoter
pAX131
Xba/Not
Amp^R
PvuI
SapI
Term
ColE1
Plus Kappa Constant Region
PvuI
f1
NheI
geneIII
stop
SfiI
SpeI
XhoI
pelB
RBS
NotI
SacI
Kappa
Constant
XbaI
ompA
SfiI
RBS
EcoRI
lac
promoter
pAX131
Kappa
Amp^R
PvuI
SapI
Term
ColE1

FIG. 18

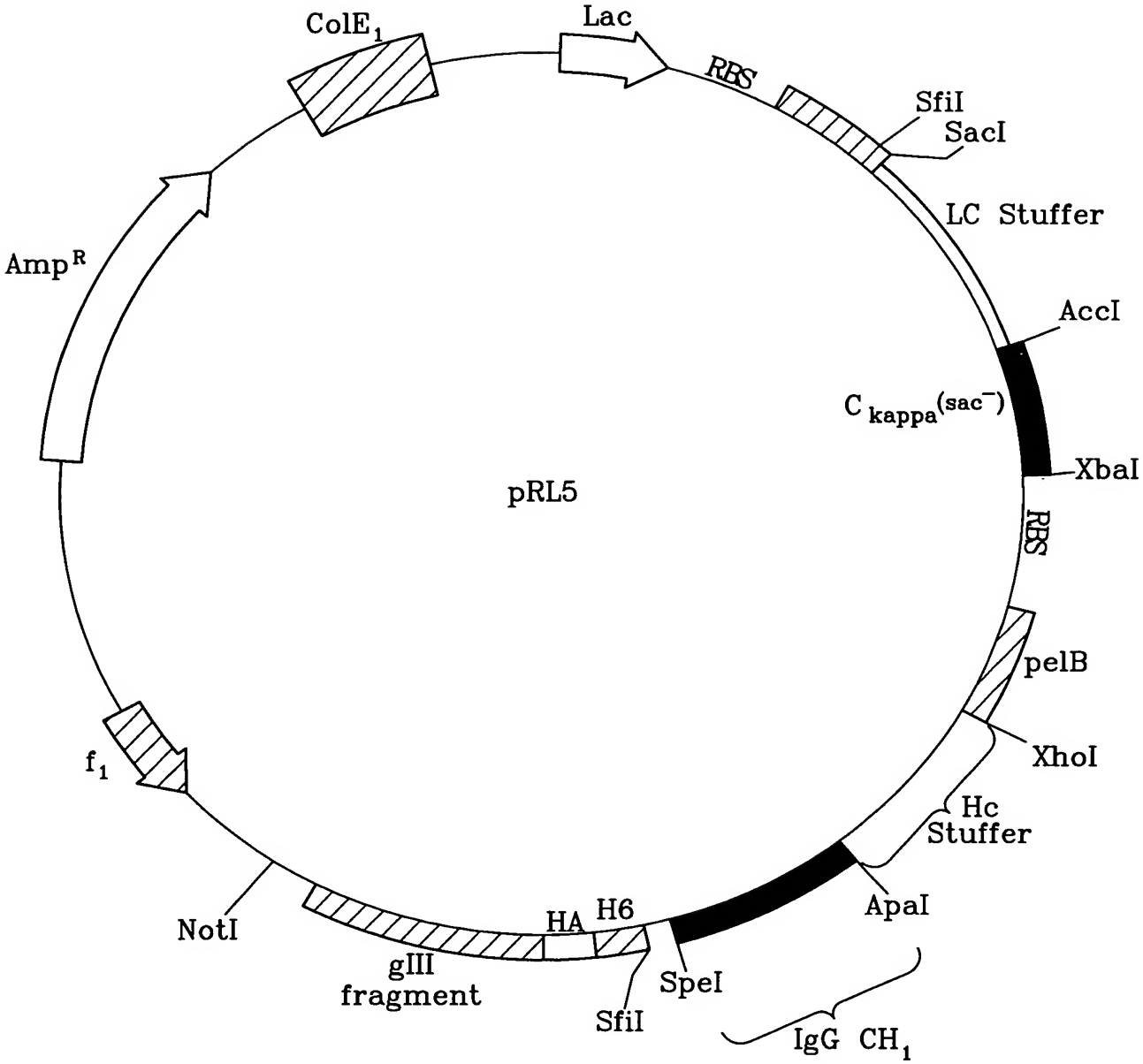

ColE1
Lac
RBS
SfiI
SacI
LC Stuffer
AccI
Ckappa(sac−)
XbaI
RBS
pelB
XhoI
Hc
Stuffer
ApaI
SpeI
IgG CH1
SfiI
HA
H6
gIII
fragment
NotI
f1
AmpR
pRL5

FIG. 19

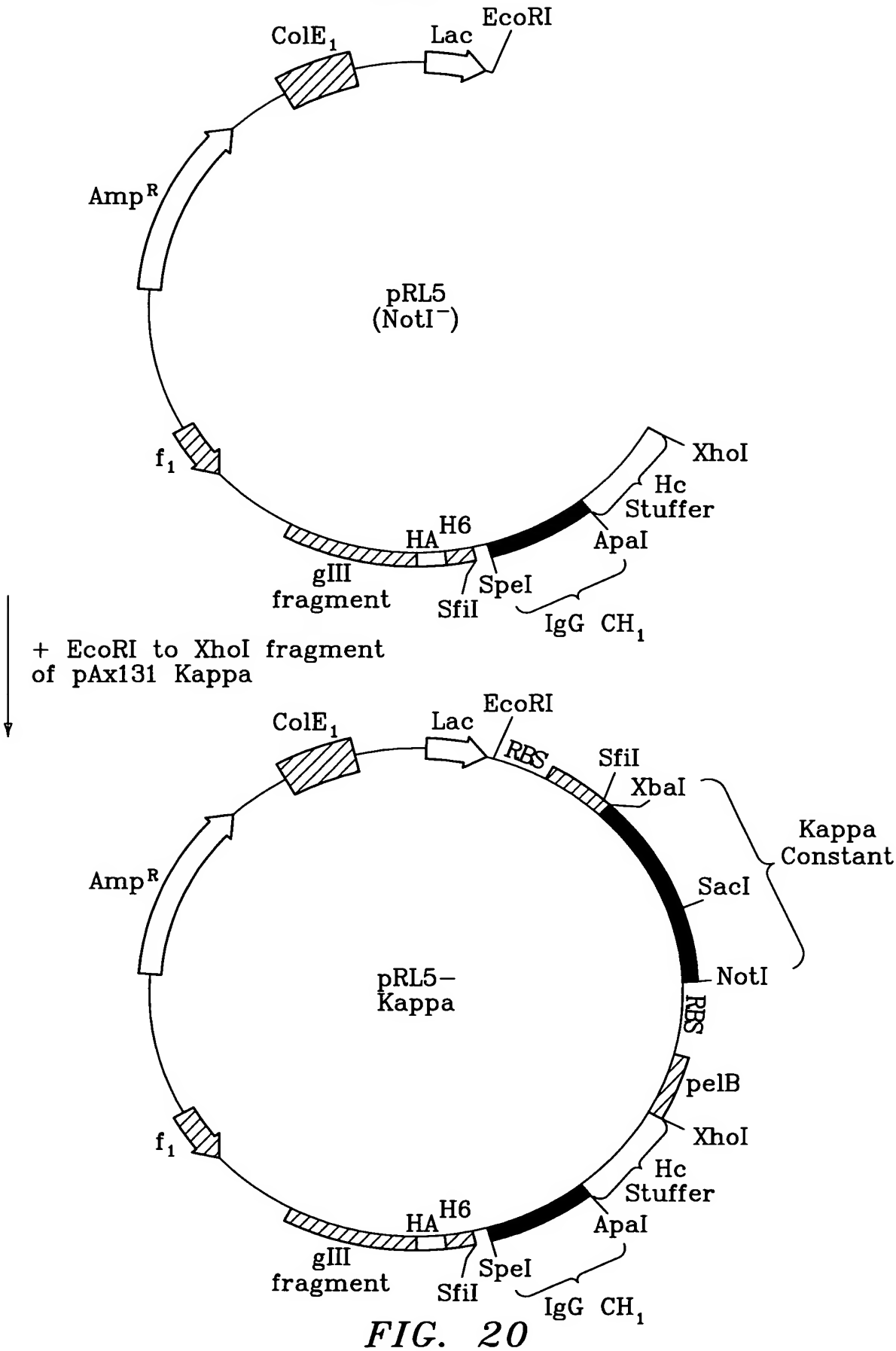

ColE1
Lac
EcoRI
Amp^R
pRL5
(NotI^-)
f1
XhoI
Hc
Stuffer
ApaI
HA
H6
gIII
fragment
SpeI
SfiI
IgG CH1
+ EcoRI to XhoI fragment
of pAx131 Kappa
ColE1
Lac
EcoRI
RBS
SfiI
XbaI
Kappa
Constant
Amp^R
SacI
pRL5-
Kappa
NotI
RBS
pelB
f1
XhoI
Hc
Stuffer
HA
H6
ApaI
gIII
fragment
SpeI
SfiI
IgG CH1

FIG. 20

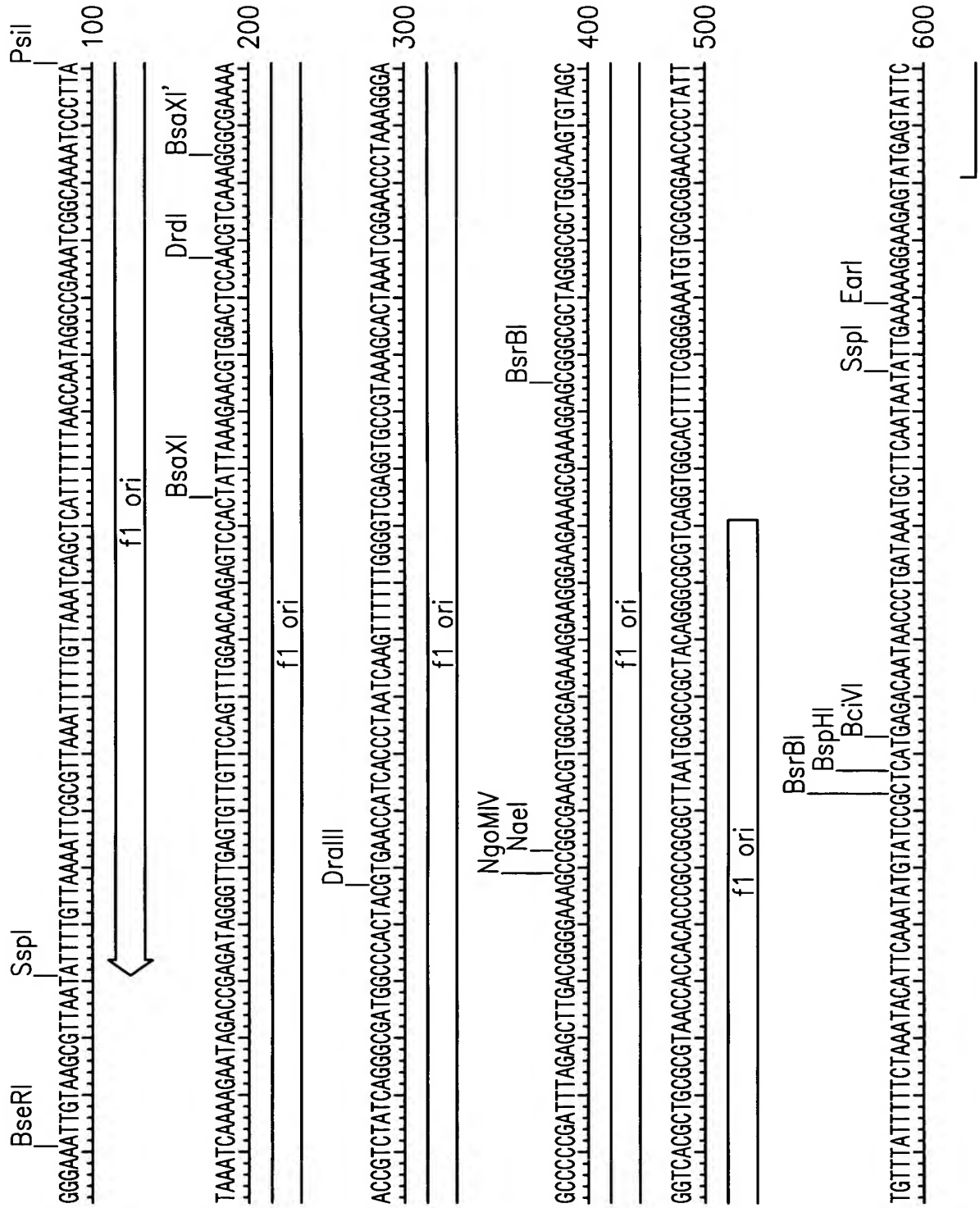

BseRI
SspI
PsiI
(SEQ ID NO: 111) GGGAAATTGTAAGCGTTAATATTTTGTTAAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTA
100
f1 ori
BsaXI
DrdI
BsaXI'
TAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAA
200
f1 ori
DraIII
ACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGA
300
f1 ori
NgoMIV
NaeI
BsrBI
GCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGC
400
f1 ori
GGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATT
500
f1 ori
BsrBI
BspHI
BciVI
SspI
EarI
TGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTC
600

FIG. 21A

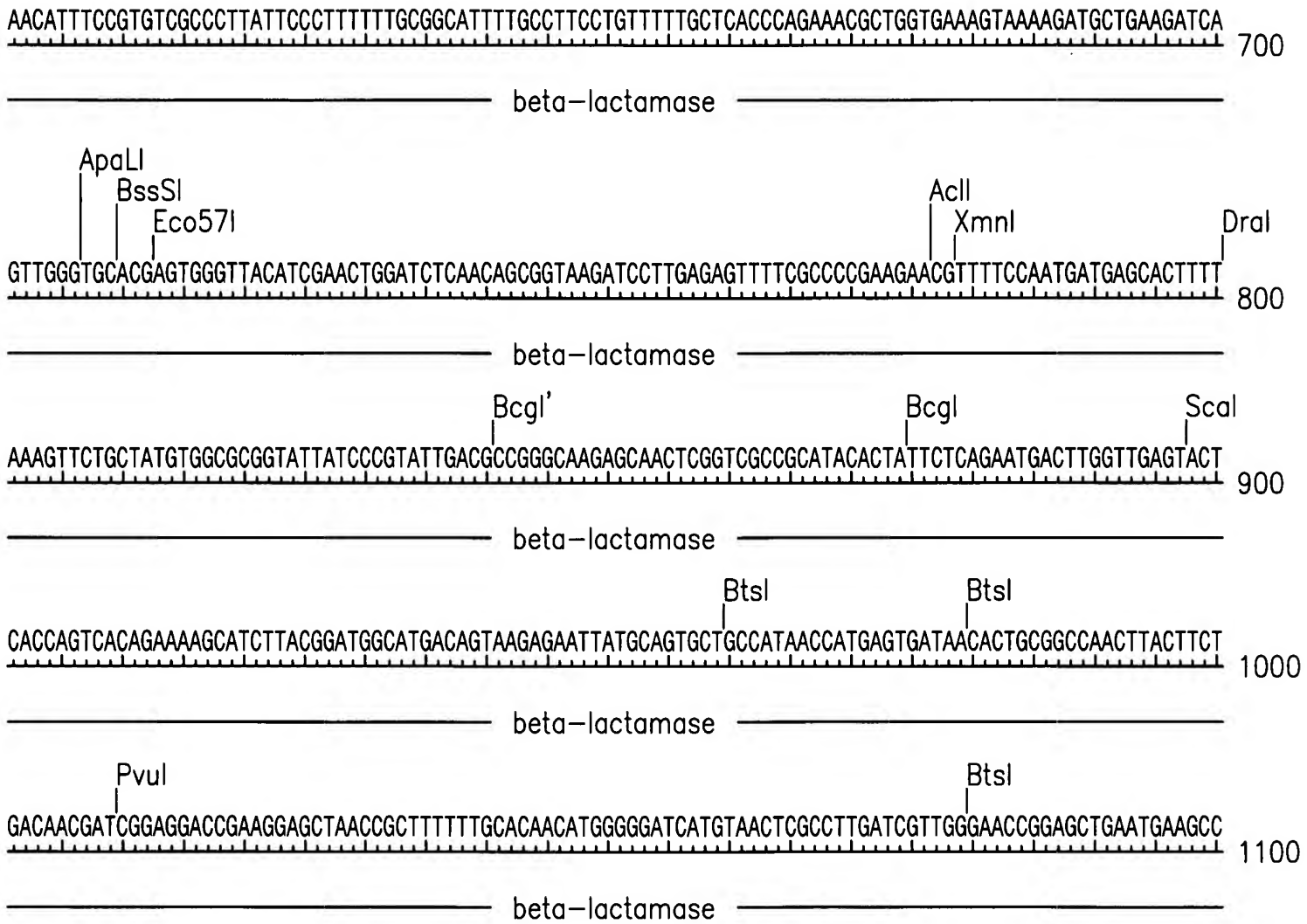

AACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCA
700
beta-lactamase
ApaLI
BssSI
Eco57I
AclI
XmnI
DraI
GTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTT
800
beta-lactamase
BcgI'
BcgI
ScaI
AAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACT
900
beta-lactamase
BtsI
BtsI
CACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCT
1000
beta-lactamase
PvuI
BtsI
GACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCC
1100
beta-lactamase

FIG. 21B

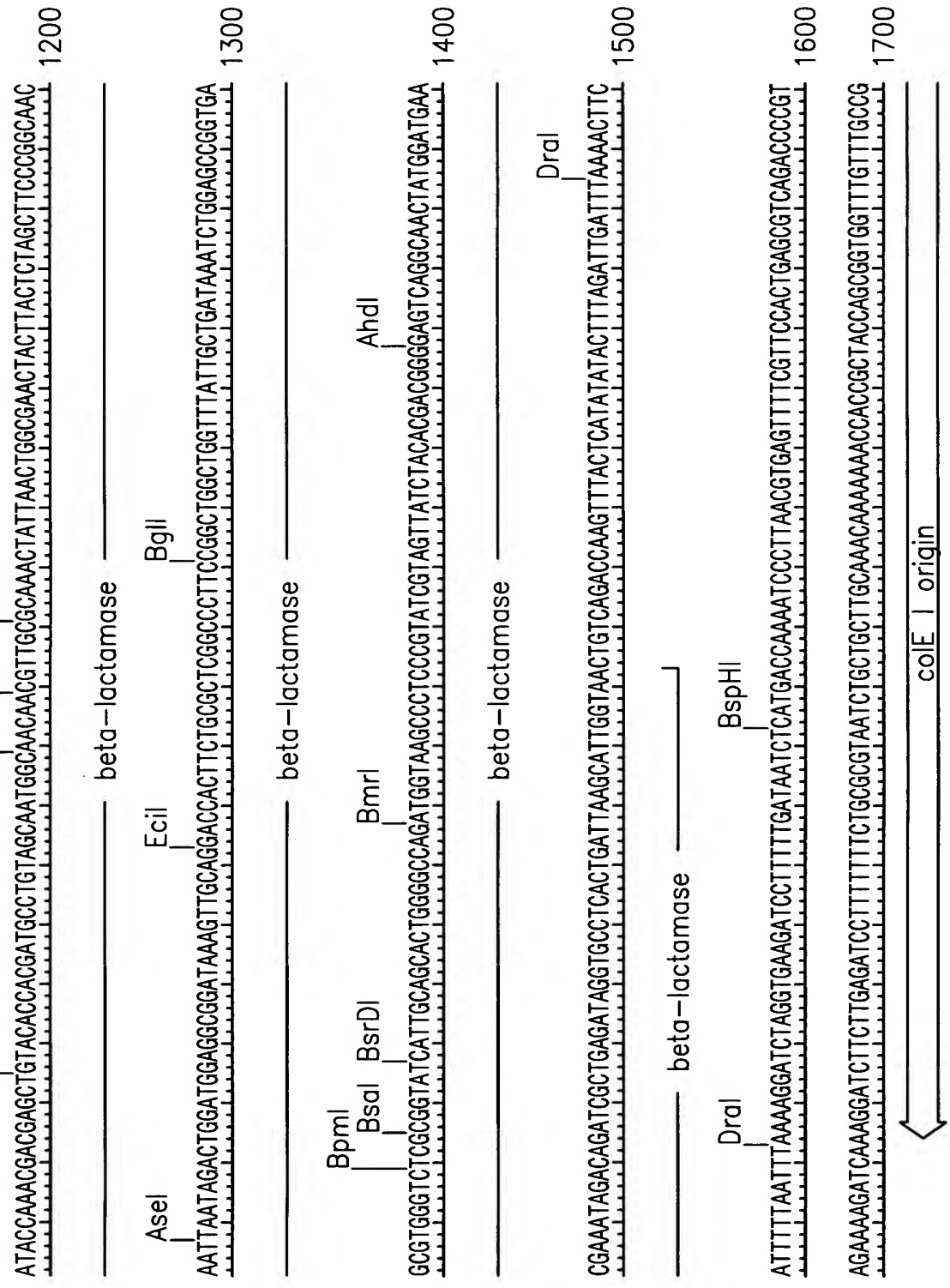

BsrDI
BsrGI
AclI
FspI
ATACCAAACGACGAGCTGTACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAAC
1200
beta-lactamase
AseI
EciI
BglI
AATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGA
1300
beta-lactamase
BpmI
BsaI
BsrDI
BmrI
AhdI
GCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAA
1400
beta-lactamase
DraI
CGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTC
1500
beta-lactamase
DraI
BspHI
ATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGT
1600
AGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCG
1700
colE1 origin

FIG. 21C

Eco57I
GATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACT
1800
colE I origin
AlwNI
TCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTC
1900
colE I origin
ApaLI
AAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATAC
2000
colE I origin
EciI BciVI
BssSI
CTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGG
2100
colE I origin
DrdI
AGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAG
2200
colE I origin
EciI
PciI
CCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTG
2300
colE I origin

FIG. 21D

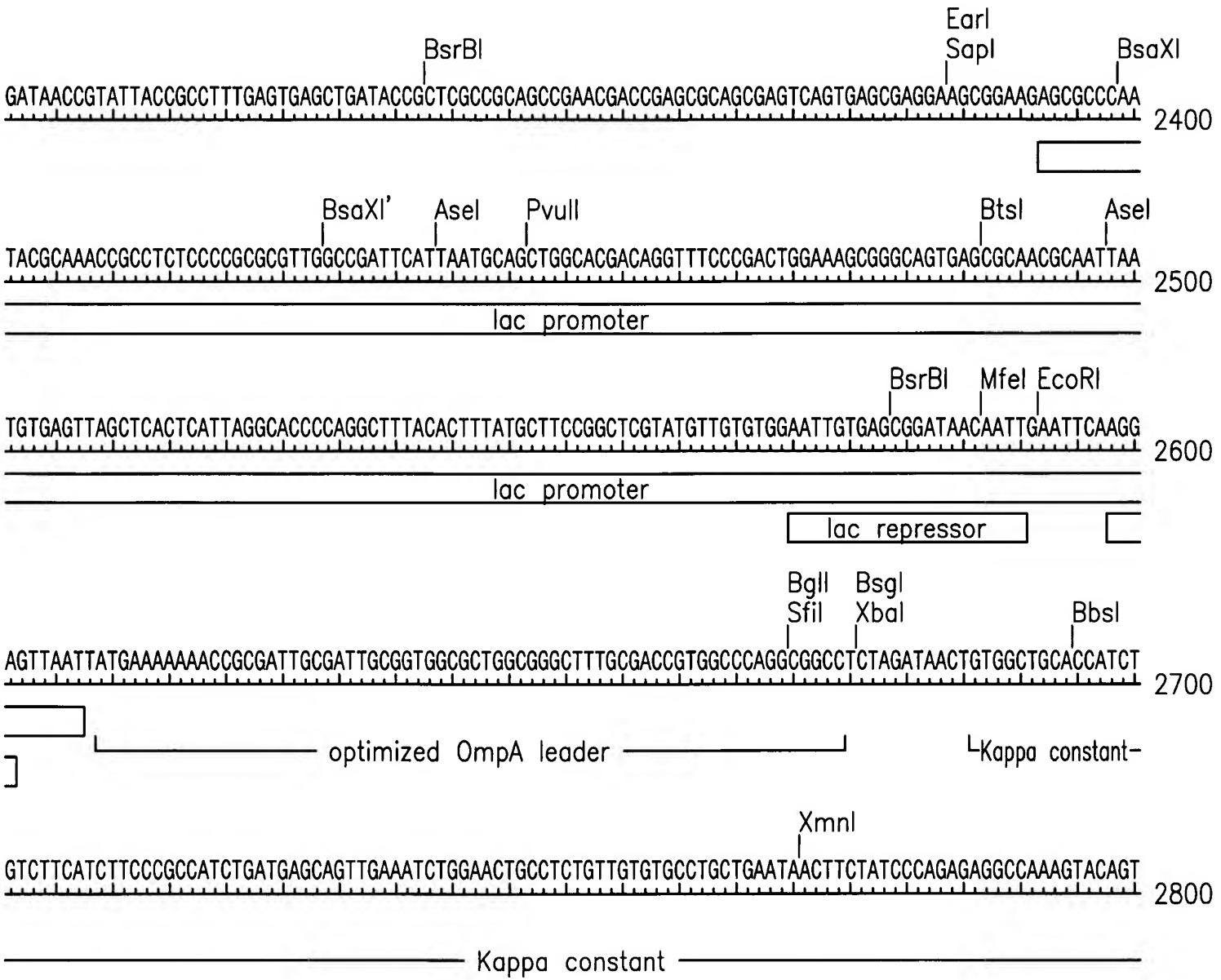

EarI
SapI
BsrBI
BsaXI
GATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAA
2400
BsaXI'
AseI
PvuII
BtsI
AseI
TACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAA
2500
lac promoter
BsrBI
MfeI
EcoRI
TGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTGAATTCAAGG
2600
lac promoter
lac repressor
BglI
SfiI
BsgI
XbaI
BbsI
AGTTAATTATGAAAAAAACCGCGATTGCGATTGCGGTGGCGCTGGCGGGCTTTGCGACCGTGGCCCAGGCGGCCTCTAGATAACTGTGGCTGCACCATCT
2700
optimized OmpA leader
Kappa constant
XmnI
GTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGT
2800
Kappa constant

FIG. 21E

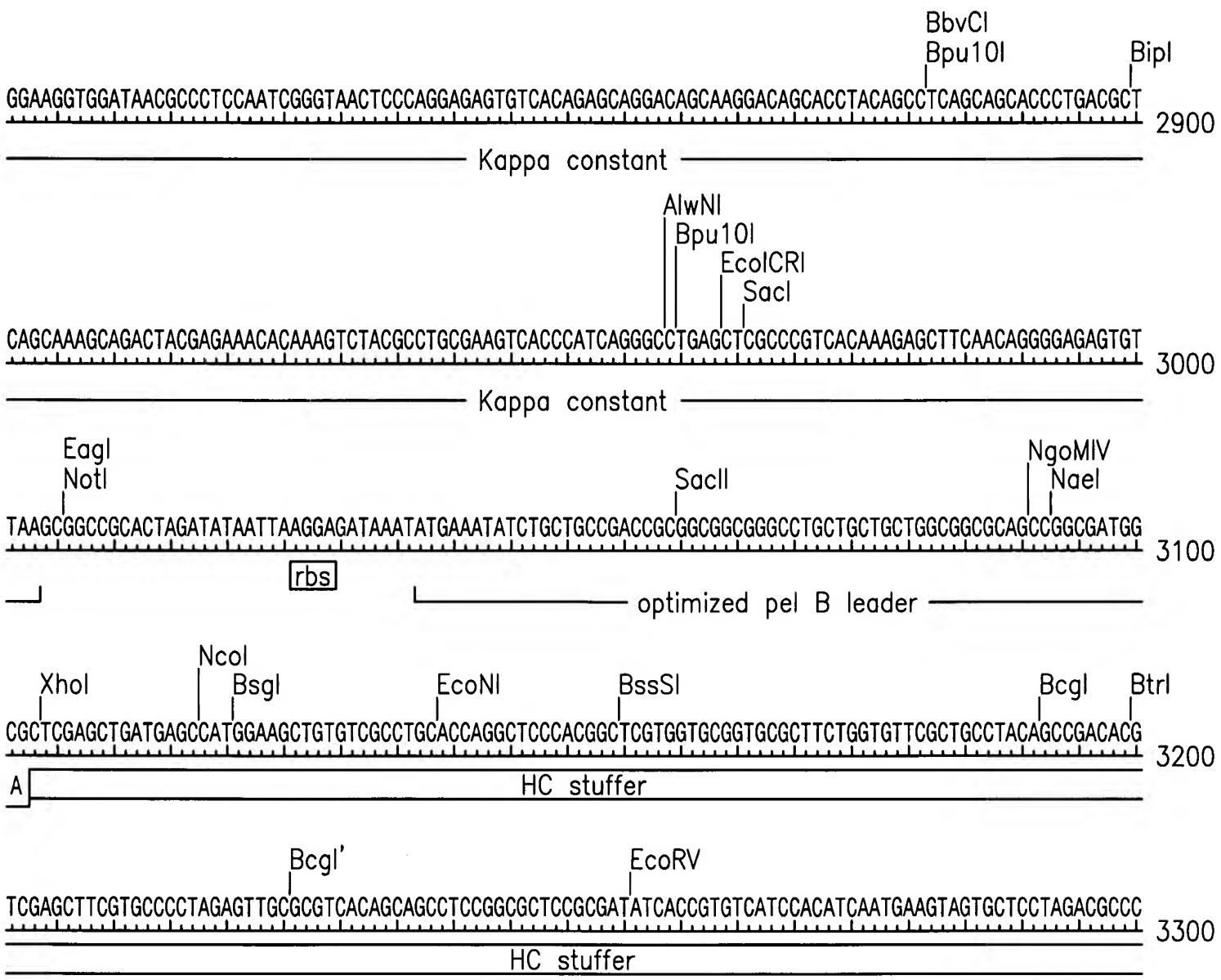

BbvCI
Bpu10I
BipI
GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCT
2900
Kappa constant
AlwNI
Bpu10I
EcoICRI
SacI
CAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
3000
Kappa constant
EagI
NotI
SacII
NgoMIV
NaeI
TAAGCGGCCGCACTAGATATAATTAAGGAGATAAATATGAAATATCTGCTGCCGACCGCGGCGGCGGGCCTGCTGCTGCTGGCGGCGCAGCCGGCGATGG
3100
rbs
optimized pel B leader
NcoI
XhoI
BsgI
EcoNI
BssSI
BcgI
BtrI
CGCTCGAGCTGATGAGCCATGGAAGCTGTGTCGCCTGCACCAGGCTCCCACGGCTCGTGGTGCGGTGCGCTTCTGGTGTTCGCTGCCTACAGCCGACACG
3200
A
HC stuffer
BcgI'
EcoRV
TCGAGCTTCGTGCCCCTAGAGTTGCGCGTCACAGCAGCCTCCGGCGCTCCGCGATATCACCGTGTCATCCACATCAATGAAGTAGTGCTCCTAGACGCCC
3300
HC stuffer

FIG. 21F

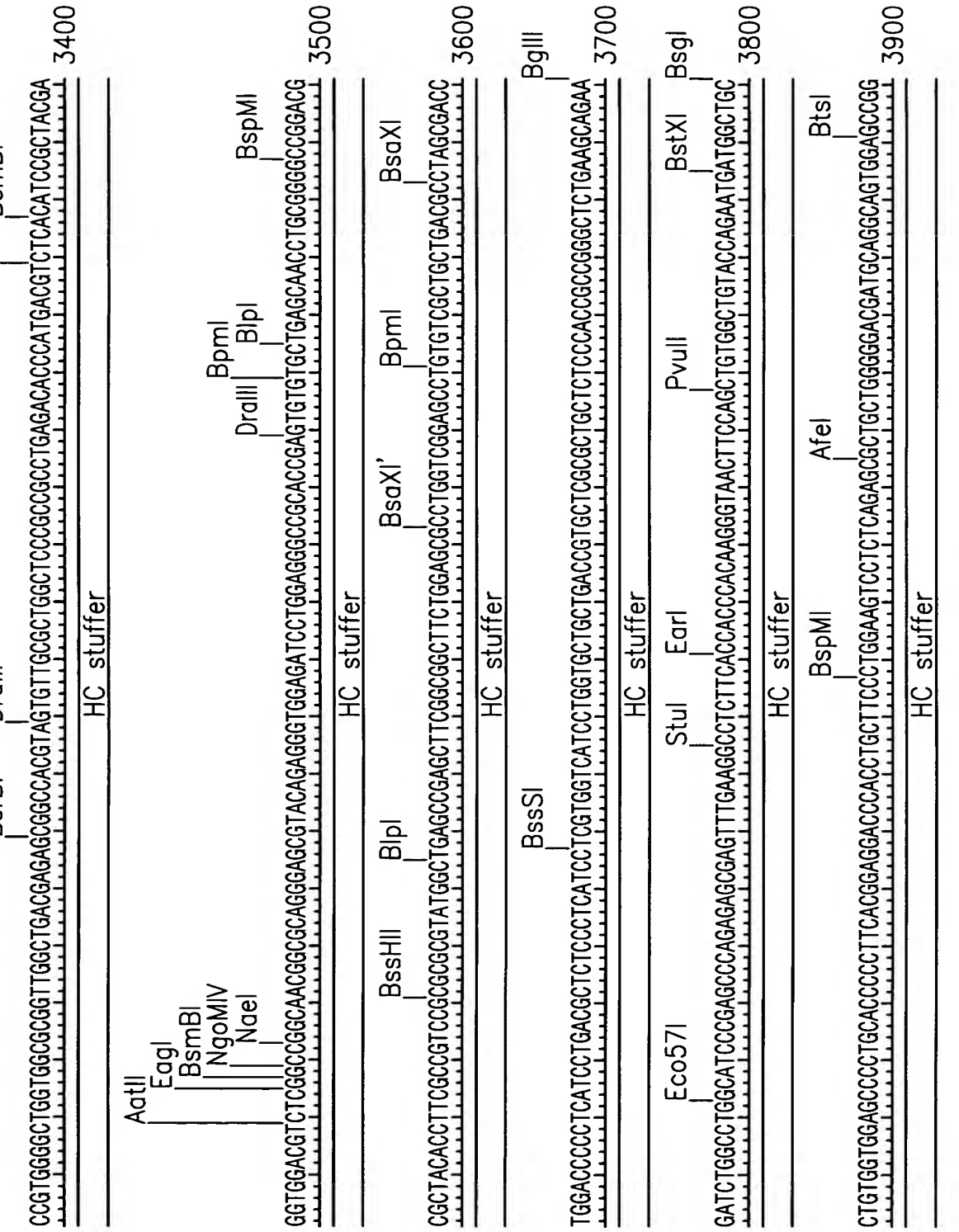

AatII
BsmBI
BsrBI
DraIII
3400
HC stuffer
AatII
EagI
BsmBI
NgoMIV
NaeI
BpmI
DraIII
BlpI
BspMI
3500
HC stuffer
BssHII
BlpI
BsaXI'
BpmI
BsaXI
3600
HC stuffer
BssSI
BglII
3700
HC stuffer
Eco57I
StuI
EarI
PvuII
BstXI
BsgI
3800
HC stuffer
BspMI
AfeI
BtsI
3900
HC stuffer

FIG. 21G

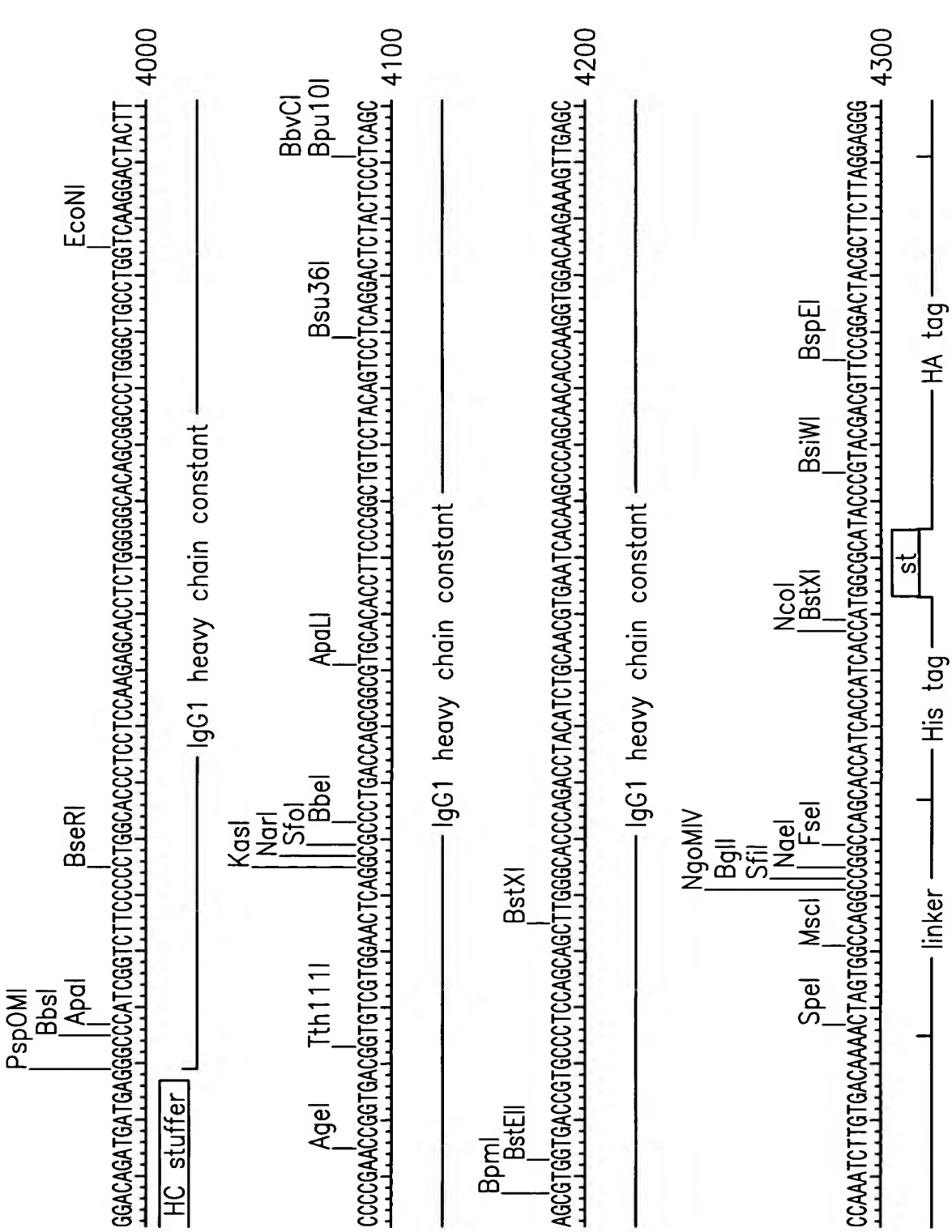

PspOMI
BbsI
ApaI
BseRI
EcoNI
GGACAGATGATGAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTT 4000
HC stuffer
IgG1 heavy chain constant
KasI
NarI
SfoI
BbvCI
AgeI
Tth111I
BbeI
ApaLI
Bsu36I
Bpu10I
CCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC 4100
IgG1 heavy chain constant
BpmI
BstEII
BstXI
AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGC 4200
IgG1 heavy chain constant
NgoMIV
BglI
SfiI
NaeI
NcoI
SpeI
MscI
FseI
BstXI
BsiWI
BspEI
CCAAATCTTGTGACAAAACTAGTGGCCAGGCCGGCCAGCACCATCACCATCACCATGGCGGCATACCCGTACGACGTTCCGGACTACGCTTCTTAGGAGGG 4300
st
linker
His tag
HA tag

FIG. 21H

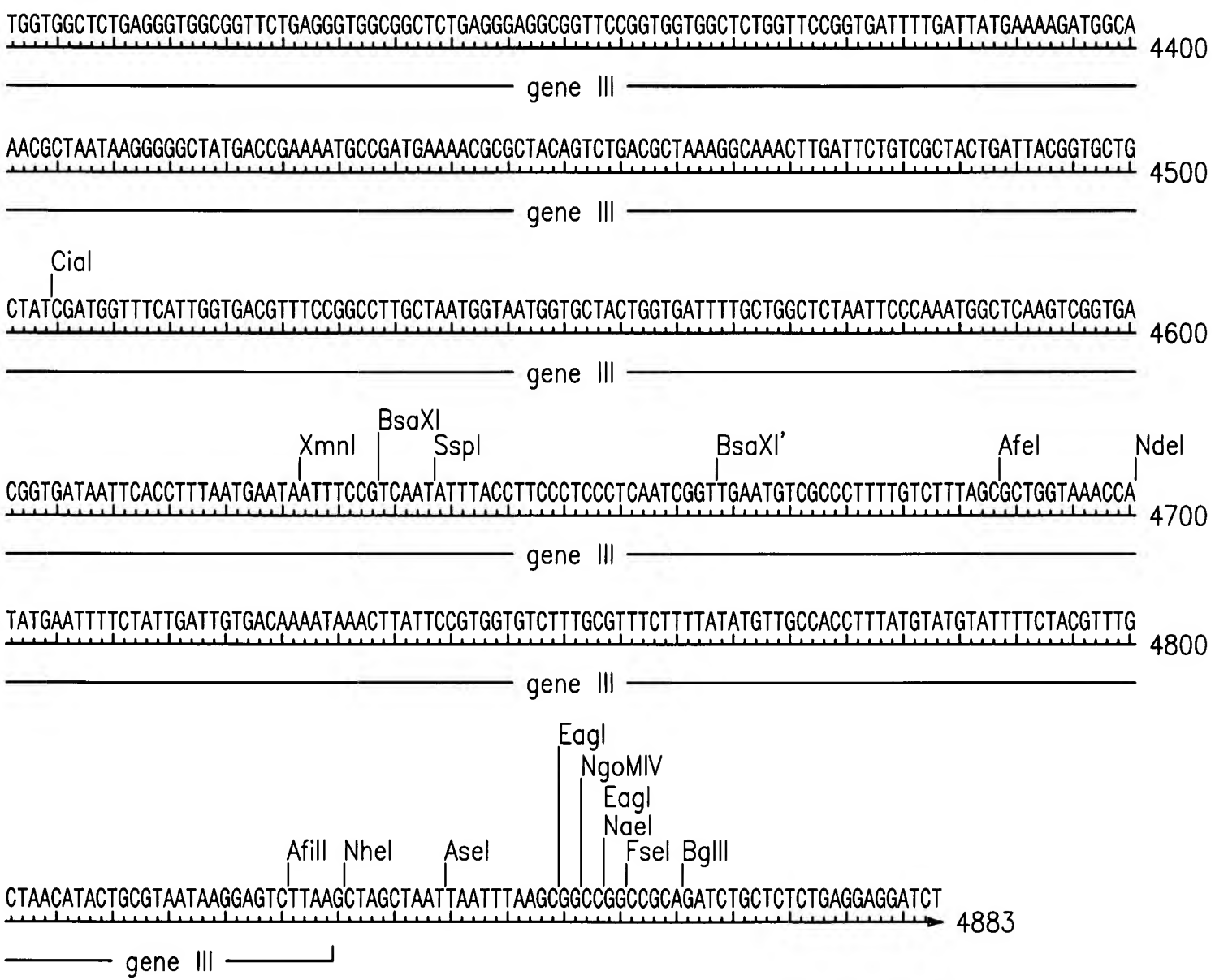

TGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGGCGGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCA
4400
gene III
AACGCTAATAAGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTCTGACGCTAAAGGCAAACTTGATTCTGTCGCTACTGATTACGGTGCTG
4500
gene III
ClaI
CTATCGATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGTGATTTTGCTGGCTCTAATTCCCAAATGGCTCAAGTCGGTGA
4600
gene III
BsaXI
XmnI
SspI
BsaXI'
AfeI
NdeI
CGGTGATAATTCACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGAATGTCGCCCTTTTGTCTTTAGCGCTGGTAAACCA
4700
gene III
TATGAATTTTCTATTGATTGTGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCACCTTTATGTATGTATTTTCTACGTTTG
4800
gene III
EagI
NgoMIV
EagI
NaeI
AflII
NheI
AseI
FseI
BglII
CTAACATACTGCGTAATAAGGAGTCTTAAGCTAGCTAATTAATTTAAGCGGCCGGCCGCAGATCTGCTCTCTGAGGAGGATCT
4883
gene III

FIG. 21I

VH: L22582 (human germline family member VH1-69)

GCAGGATTTAGGGCTTGGTCTCTCAGCATCCCACACTTGTACAGCTGATGTGGCATCTG
TGTTTTCTTTCTCATCGTAGATCAGGCTTTGAGCTGTGAAATACCCTGCCTCATGCATATGCA
AATAACCTGAGGTCTTCTGAGATAAATATAGATATATTGGTGCCCTGAGAGCATCACATAA
CAACCACATTCCTCCTCTAAAGAAGCCCCTGGGAGCACAGCTCATCACCATGGACTGGACC
TGGAGGTTCCTCTTTGTGGTGGCAGCGCTACAGGTAAGGGGCTTCCTAGTCCTAAGGCTGAG
GAAGGGATCCTGGTTTAGTTAAAGAGGATTTTATTCACCCCTGTGTCCTCTCCACAGGTGTC
CAGTCCCAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGA
AGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCGACA
GGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAAC
TACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGCCT
ACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGACAC
AGTGTGAAAACCCACATCCTGAGAGTGTCAGAAACCCTGAGGGAGAAGGCAGCTGTGCCG
GGCTGAGGAGATGACAGGGTTTATTAGGTTTAAGGCTGTTTACAAAATGGGTTATATATTTG
AGAAAAAAAGAACAGTAGAAACAAGTACATACTCCTCTAATTTTAAGATAATTATTCCATT
CAAGAGTCGTAATAT (SEQ ID NO: 153)

JH---JH6

H3

CDR3

100 110

JH6 YYYYYGMDVWGQGTTVTVSS (SEQ ID NO: 154)

FIG. 22

Vk: X12686 (human germline family member VKIII-A27)

CAGCTGCTTTGCATGTCCCTCCCAGCCGCCCTGCAGTCCAGAGCCCATATCAATGCCTGG
GTCAGAGCTCTGGAGAAGAGCTGCTCAGTTAGGACCCAGAGGGAACCATGGAAACCCCAG
CGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAGGTGAGGGGAACATGGGATGGTTTTGC
ATGTCAGTGAAAACCCTCTCAAGTCCTGTTACCTGGCAACTCTGCTCAGTCAATACAATAAT
TAAAGCTCAATATAAAGCAATAATTCTGGCTCTTCTGGGAAGACAATGGGTTTGATTTAGAT
TACATGGGTGACTTTTCTGTTTTATTTCCAATCTCAGATACCACCGGAGAAATTGTGTTGAC
GCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCA
GTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAG
GCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGT
GGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGT
ATTACTGTCAGCAGTATGGTAGCTCACCTCCCACAGTGATTCAGCTTGAAACAAAAACCTCT
GCAAGACCTTCATTGTTTACTAGATTATACCAGCTG (SEQ ID NO: 155)

JK

L3

CDR3

100

Jk1 WTFGQGTKVEIK (SEQ ID NO: 156)

FIG. 23

pAXB116 Fab' gVh

(SEQ ID NO: 157)

pelB leader

```
ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA
M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L   A   A   Q
```

(SEQ ID NO: 158)

pelB leader

```
CCA GCC ATG GCG CAG GTG CAG CTG GTG CAG AGC GGC GCG GAA GTG AAA AAA CCG
P   A   M   A   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   pAXB116 Fab'-gVh
                (SEQ ID NO: 159)    Q   S   G   A   E   V   K   K   P   TT-Vh(CDR3-TPO)
(SEQ ID NO: 169)    Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   L22582
```

CDR1-H1

```
GGC AGC AGC GTG AAA GTG AGC TGC AAA GCG AGC GGC GGC ACC TTT AGC AGC TAT
G   S   S   V   K   V   S   C   K   A   S   G   G   T   F   S   S   Y   pAXB116 Fab'-gVh
G   S   S   V   K   V   S   C   K   A   S   G   G   T   F   N   N   Y   TT-Vh(CDR3-TPO)
G   S   S   V   K   V   S   C   K   A   S   G   G   T   F   S   S   Y   L22582

GCG ATT AGC TGG GTG CGC CAG GCG CCG GGC CAG GGC CTG GAA TGG ATG GGC CAG
A   I   S   W   V   R   Q   A   P   G   Q   G   L   E   W   M   G   Q   pAXB116 Fab'-gVh
A   I   S   W   V   R   Q   A   P   G   Q   G   L   E   W   M   G   G   TT-Vh(CDR3-TPO)
A   I   S   W   V   R   Q   A   P   G   Q   G   L   E   W   M   G   G   L22582
```

CDR2-TPO

```
CTG ATT GAA GGC CCG ACC CTG CGC CAG TGG CTG GCG GCG CGC GCG AAC AGC CGC
L   I   E   G   P   T   L   R   Q   W   L   A   A   R   A   N   S   R   pAXB116 Fab'-gVh
    I   F   P   F   R   N   T   A   K   Y   A   Q   H   F   Q   G   R   TT-Vh(CDR3-TPO)
I   I   P   I   F   G   T   A   N       Y   A   Q   K   F   Q   G   R   L22582

GTG ACC ATT ACC GCG GAT GAA AGC ACC AGC ACC GCG TAT ATG GAA CTG AGC AGC
V   T   I   T   A   D   E   S   T   S   T   A   Y   M   E   L   S   S   pAXB116 Fab'-gVh
V   T   I   T   A   D   E   S   T   G   T   A   Y   M   E   L   S   S   TT-Vh(CDR3-TPO)
V   T   I   T   A   D   E   S   T   S   T   A   Y   M   E   L   S   S   L22582

CTG CGC AGC GAA GAT ACC GCG GTG TAT TAT TGC GCG CGC CTG CCG ATT GAA GGC
L   R   S   E   D   T   A   V   Y   Y   C   A   R   L   P   I   E   G   pAXB116 Fab'-gVh
L   R   S   E   D   T   A   I   Y   Y   C   A   R   L   P   I   E   G   TT-Vh(CDR3-TPO)
L   R   S   E   D   T   A   V   Y   Y   C   A   R                       L22582
```

CDR3-TPO

```
CCG ACC CTG CGC CAG TGG CTG GCG GCG CGC GCG CCG GTG TGG GGC CAG GGC ACC
P   T   L   R   Q   W   L   A   A   R   A   P   V   W   G   Q   G   T   pAXB116 Fab'-gVh
P   T   L   R   Q   W   L   A   A   R   A   P   V   W   G   Q   G   T   TT-Vh(CDR3-TPO)

ACC GTG ACC GTG AGC AGC
T   V   T   V   S   S                                                   pAXB116 Fab'-gVh
T   V   T   V   S   A                                                   TT-Vh(CDR3-TPO)
```

Sequence of the pAXB116 Fab' Heavy chain variable region. The cDNA sequence with the best E. coli codon usage (Henaut and Danchin, 1996) and the translated amino acid sequence of pAXB116 Fab' are shown. CDR (Complementarity Determining Region) are defined by Kabat et al(1992) and the structural variability definition sequence (Chothia and Lesk, 1987) underlined and overlined, respectively. TPO peptides in heavy chain CDR2 and CDR3 of pAXB116 Fab' are indicated by double underlines and wavelines respectively. pelB leader cDNA sequences are overlined. pAXB116 Fab'-gVh denotes heavy chain variable region of human germline derived pAXB116 Fab'.

FIG. 24

pAXB116 Fab' gVk

(SEQ ID NO: 160)

pelB leader

```
ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA
M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L   A   A   Q
```

(SEQ ID NO: 161)

pelB leader

```
CCA GCC ATG GCG GAA ATT GTG CTG ACC CAG AGC CCG GGC ACC CTG AGC CTG AGC
P   A   M   A   E   I   V   L   T   Q   S   P   G   T   L   S   L   S   pAXB116 Fab'-gVk
    (SEQ ID NO: 162) E   L   T   Q   S   P   G   T   L   S   L   S   TTVk
(SEQ ID NO: 170) E   I   V   L   T   Q   S   P   G   T   L   S   L   S   X12686
```

CDR1-L1

```
CCG GGC GAA CGC GCG ACC CTG AGC TGC CGC GCG AGC CAG AGC GTG AGC AGC AGC
P   G   E   R   A   T   L   S   C   R   A   S   Q   S   V   S   S   S   pAXB116 Fab'-gVk
P   G   E   R   A   T   L   S   C   R   A   S   H   S   V   S   R   A   TTVk
P   G   E   R   A   T   L   S   C   R   A   S   Q   S   V   S   S   S   X12686

TAT CTG GCG TGG TAT CAG CAG AAA CCG GGC CAG GCG CCG CGC CTG CTG ATT TAT
Y   L   A   W   Y   Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   pAXB116 Fab'-gVk
Y   L   A   W   Y   Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   TTVk
Y   L   A   W   Y   Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   X12686
```

CDR2-L2

```
GGC GCG AGC AGC CGC GCG ACC GGC ATT CCG GAT CGC TTT AGC GGC AGC GGC AGC
G   A   S   S   R   A   T   G   I   P   D   R   F   S   G   S   G   S   pAXB116 Fab'-gVk
G   T   S   S   R   A   T   G   I   P   D   R   F   S   G   S   G   S   TTVk
G   A   S   S   R   A   T   G   I   P   D   R   F   S   G   S   G   S   X12686

GGC ACC GAT TTT ACC CTG ACC ATT AGC CGC CTG GAA CCG GAA GAT TTT GCG GTG
G   T   D   F   T   L   T   I   S   R   L   E   P   E   D   F   A   V   pAXB116 Fab'-gVk
G   T   D   F   T   L   T   I   S   R   L   E   P   E   D   F   A   V   TTVk
G   T   D   F   T   L   T   I   S   R   L   E   P   E   D   F   A   V   X12686
```

CDR3-L3

```
TAT TAT TGC CAG CAG TAT GGC AGC AGC CCG TGG ACC TTT GGC CAG GGC ACC AAA
Y   Y   C   Q   Q   Y   G   S   S   P   W   T   F   G   Q   G   T   K   pAXB116 Fab'-gVk
Y   Y   C   Q   Q   Y   G   G   S   P   W       F   G   Q   G   T   K   TTVk
Y   Y   C   Q   Q   Y   G   S   S   P                                   X12686

GTG GAA ATT AAA
V   E   I   K                                                           pAXB116 Fab'-gVk
V   E   L   K                                                           TTVk
```

Sequence of the pAXB116 Fab' Light chain variable region. The cDNA sequence with the best E. coli codon usage (Henaut and Danchin, 1996) and the translated amino acid sequence of pAXB116 Fab' are shown. CDR (Complementarity Determining Region) are defined by Kabat et al(1992) and the structural variability definition sequence (Chothia and Lesk, 1987) underlined and overlined, respectively. pelB leader cDNA sequences are overlined. pAXB116 Fab'-gVk denotes light chain variable region of human germline derived pAXB116 Fab'.

FIG. 25

Primers to generate pAXB116 heavy chain

UDEC1709 :5' primer 272 bp, containing NcoI site (SEQ ID NO: 163)

5'----CCAG*CCATGG*CGCAGGTGCAGCTGGTGCAGAGCGGCGCGGAAGTGAAAAAACCGGGCAGCAGCGTGAAAGT
GAGCTGCAAAGCGAGCGGCGGCACCTTTAGCAGCTATGCGATTAGCTGGGTGCGCCAGGCGCCGGGCCAGGGCCTG
GAATGGATGGGCGGCATTATTCCGATTTTGGCACCGCGAACTATGCGCAGAAATTTCAGGGCCGCGTGACCATTAC
CGCGGATGAAAGCACCAGCACCGCGTATATGGAACTGAGCAGCCTGCG---3'

Overlapping with UDEC1710

UDEC1710 3' primer 271 bp

5'----GTTCCAGCTCACGGTCACCGGTTCCGGAAAATAATCTTTCACCAGGCAGCCCAGCGCCGCGGTGCCGCCG

Overlapping with UDEC1711

CTGGTGCTTTTGCTGCTCGGCGCCAGCGGAAACACGCTCGGGCCTTTGGTGCTCGCGCTGCTCACGGTCACGGTGGT
GCCCTGGCCCCACACCGGCGCGCGCGCCGCCAGCCACTGGCGCAGGGTCGGGCCTTCAATCGGCAGGCGCGCGCAA
TAATACACCGCGGTATCTTCGCTGCGCAGGCTGCTCAGTTCCATATAC----3' (SEQ ID NO: 164)

Overlapping with UDEC1709

UDEC1711 3' primer (274 bp) containing XbaI site

5'----CGAG*TCTAGA*TTACGGGCCGCCCAGCAGTTCCGGCGCCGGGCACGGCGGGCAGGTATGGGTTTTATCGCAGCT
TTTCGGTTCCACTTTTTTATCCACTTTGGTGTTGCTCGGTTTATGGTTCACGTTGCAAATATAGGTCTGGGTGCCCAGG
CTGCTGCTCGGCACGGTCACCACGCTGCTCAGGCTATACAGGCCGCTGCTCTGCAGCACCGCCGGAAAGGTATGCAC
GCCGCTGGTCAGCGCGCCGCTGTTCCAGCTCACGGTCACCGGTTC----3' (SEQ ID NO: 165)

Overlapping with UDEC1710

FIG. 26

Primers to generate pAXB116 light chain

UDEC1712 5' primer 236 bp

5'---CCAGCCATGGCGGAAATTGTGCTGACCCAGAGCCCGGGCACCCTGAGCCTGAGCCCGGGCGAACGCGCGAC
CCTGAGCTGCCGCGCGAGCCAGAGCGTGAGCAGCAGCTATCTGGCGTGGTATCAGCAGAAACCGGGCCAGGCGCCG
CGCCTGCTGATTTATGGCGCGAGCAGCCGCGCGACCGGCATTCCGGATCGCTTTAGCGGCAGCGGCAGCGGCACCG
ATTTTACCCTGAC---3' (SEQ ID NO: 166) Overlapping with UDEC1713 (24bp)

UDEC1713 3' primer 239 bp

5'---CTTTCGCTTCGCGCGGATAAAAGTTGTTCAGCAGGCACACCACGCTCGCGGTGCCGCTTTTCAGTGTTCA

Overlapping with UDEC1714

TCGCTCGGCGGAAAAATAAACACGCTCGGCGCCGCCACGGTGCGTTTAATTTCCACTTTGGTGCCCTGGCCAAAGGT
CCACGGGCTGCTGCCATACTGCTGGCAATAATACACCGCAAAATCTTCCGGTTCCAGGCGGCTAATGGTCAGGGTAA
AATCGGTGCCGCTG----3' (SEQ ID NO: 167) Overlapping with UDEC1712 (24bp)

UDEC1714 3' primer 245 bp

5'----GTGCTGATCATTAGCATTCGCCGCGGTTAAAGCTTTTGGTCACCGGCAGGCTCAGGCCCTGATGGGTCACTTC
GCACGCATACACTTTATGTTTTTCATAATCCGCTTTGCTCAGGGTCAGGGTGCTGCTCAGGCTATAGGTGCTATCTTT
GCTATCCTGTTCGGTCACGCTTTCCTGGCTGTTGCCGCTCTGCAGCGCGTTATCCACTTTCCACTGCACTTTCGCTTCG
CGCGGATAAAAGTTG---3' (SEQ ID NO: 168) Overlapping with UDEC1713 (26bp)

FIG. 27

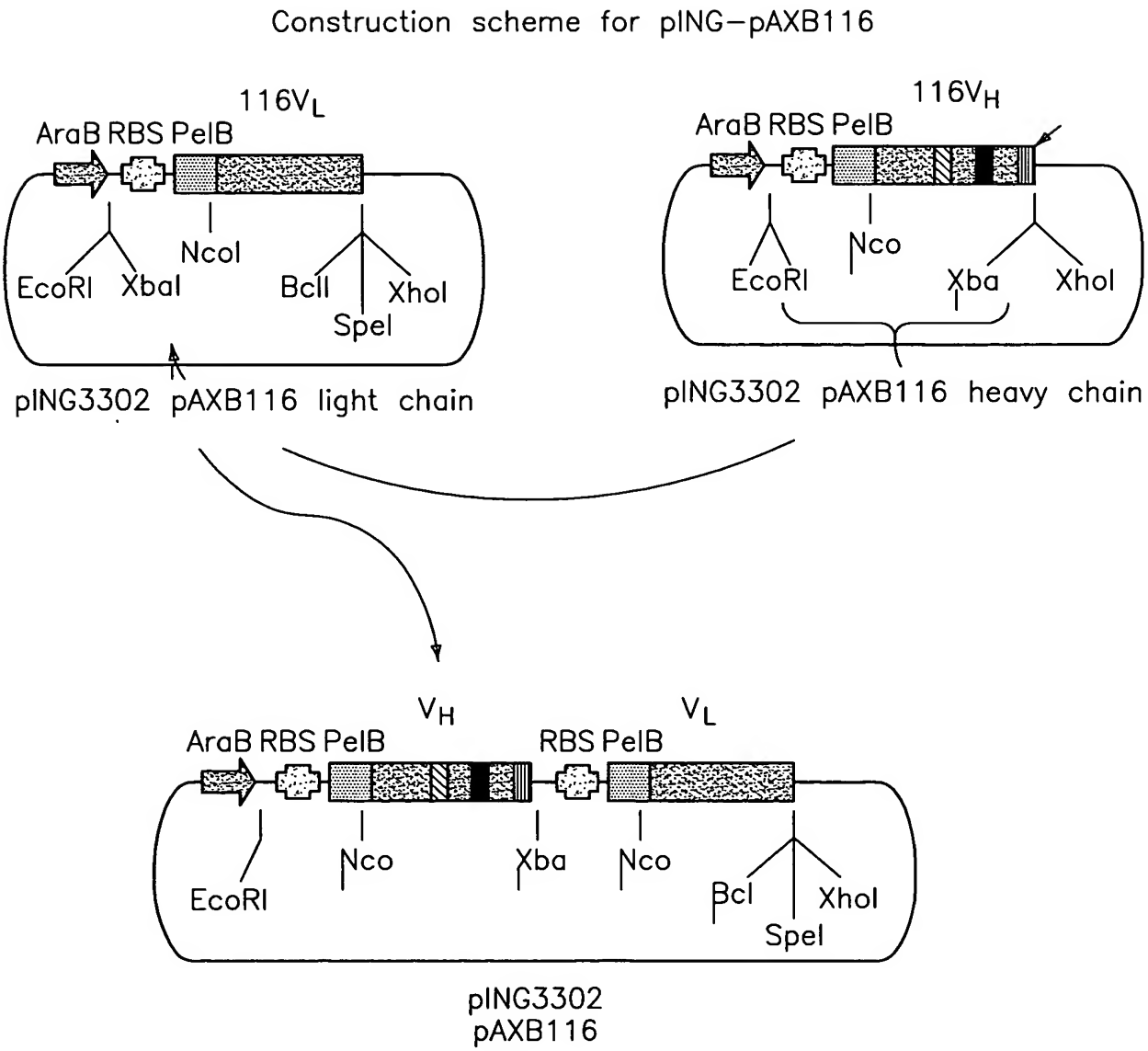

Construction scheme for pING−pAXB116
116VL
AraB RBS PelB
EcoRI
XbaI
NcoI
BclI
SpeI
XhoI
pING3302 pAXB116 light chain
116VH
AraB RBS PelB
EcoRI
Nco
Xba
XhoI
pING3302 pAXB116 heavy chain
VH
VL
AraB RBS PelB
RBS PelB
EcoRI
Nco
Xba
Nco
Bcl
XhoI
SpeI
pING3302
pAXB116

FIG. 28

116 Light Chain (SEQ. ID NO. 122):

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSLPVTKSFNRGEC.

Variable Region of 116 Light Chain (SEQ. ID NO. 123):

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK

116 Heavy Chain (SEQ. ID NO. 124):

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGQLIEGPTLRQWLA
ARANSRVTITADESTSTAYMELSSLRSEDTAVYYCARLPIEGPTLRQWLAARAPVWGQGTTVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK **{VEPKSCDKTHTCPPCP}** *APELLGGP*

end CH1 constant domain — hinge region — tail region

Variable Region of 116 Heavy Chain (SEQ. ID NO. 125):

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGQLIEGPTLRQWLA
ARANSRVTITADESTSTAYMELSSLRSEDTAVYYCARLPIEGPTLRQWLAARAPVWGQGTTVTV
SS

Clone 116. The light chain sequence is as given. The heavy chain may have several forms depending on the final antibody form. For exmaple: the minimum sequence ending with the CH1 constant domain may result in Fab assembly but the heavy chain/light chain interaction will not be stable. Commonly, a portion of the hinge region containing a cysteine (the underlined bold portion of hinge region) may be included for a covalent interaction between the heavy and light chains. Fab'_2 association would need further cysteines such as an entire IgG_1 hinge region (bold). In this example, clone 116 was cloned in a Xoma pING3302 modified vector which includes a transition tail region (italicized).

FIG. 29

SDS–PAGE of pAXB116, culture supernatant was resolved from each other by non–reducing 4–12% SDS–PAGE (lane "Sup") and cell lysate by reducing 4–12% SDS–PAGE (lane "Lysate"). Proteins were transferred onto Hybond Nitrocellulose Sheet (Amersham) and block with TBS–0.2% Tween–20+10% (w/v) Carnation nonfat dry milk. PAXb116 were detected by HRP–conjugated goat–antiHuman (H+L)Ab (Chenicon Cat#AP112P, (Temecula, CA). The signals were detected by ECL.

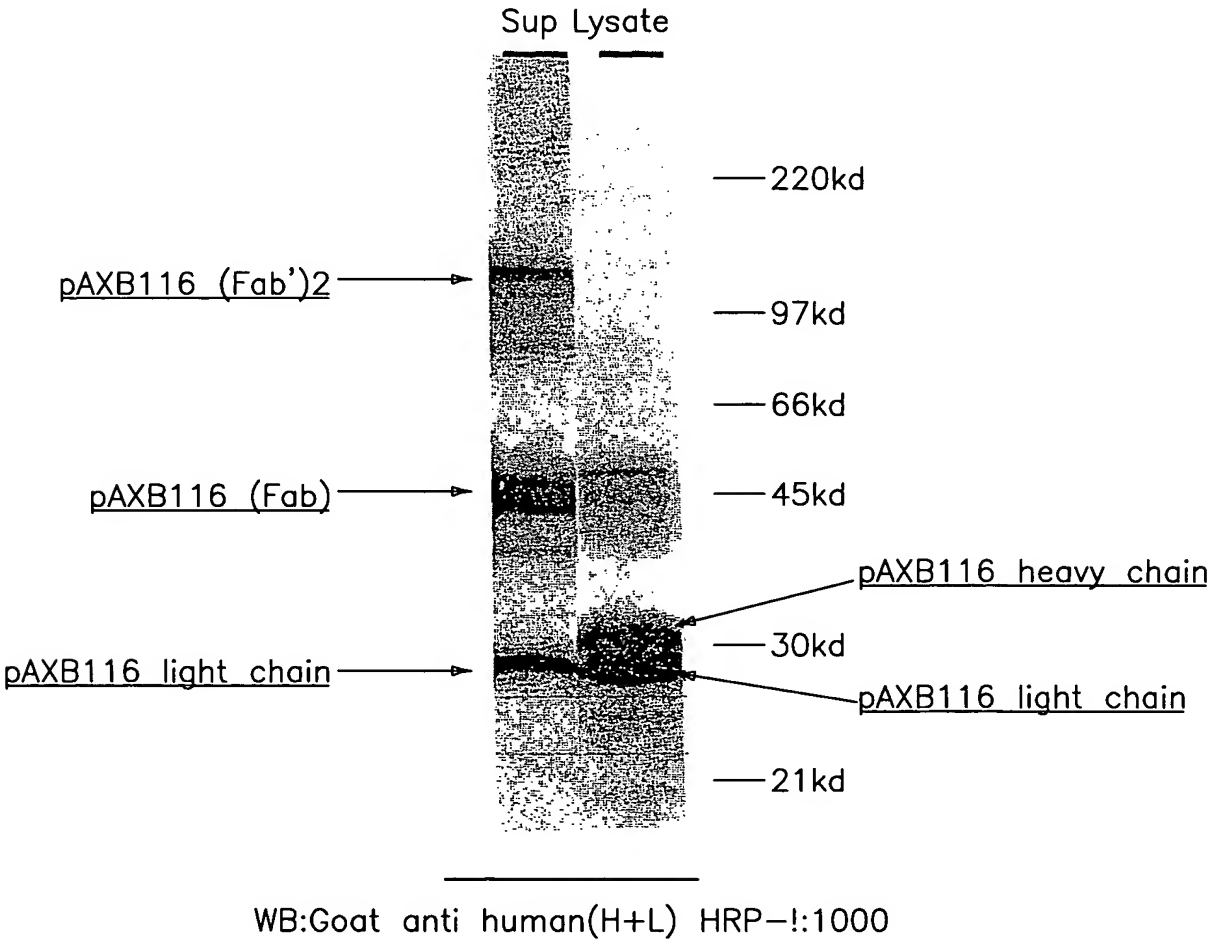


FIG. 30

$CD34^+$ cord blood cells (Poeisis) were thawed, washed, resuspended in BIT9500 serum–substituted medium (StemCell Technologies, Inc.), and plated at 3.5 x 10^5 per well in a 96 well flat–bottom plate with increasing concentrations of either recombinant human TPO (R&D Systems), circle, or pAXB116, square. After four days of culture at 37°C in a 5% CO_2 incubator, 1 Ci of ^3H thymidine (Perkin Elmer) was added to each well and cells were further incubated for 16 hours. Cells were harvested with an automatic 96–well cell harvester. ^3H incorporation was measured using a betaplate liquid scintillation counter (Wallac). Proliferation of cord blood cells are measured as counts per minute (CPM) and CPM values are an average of three wells.

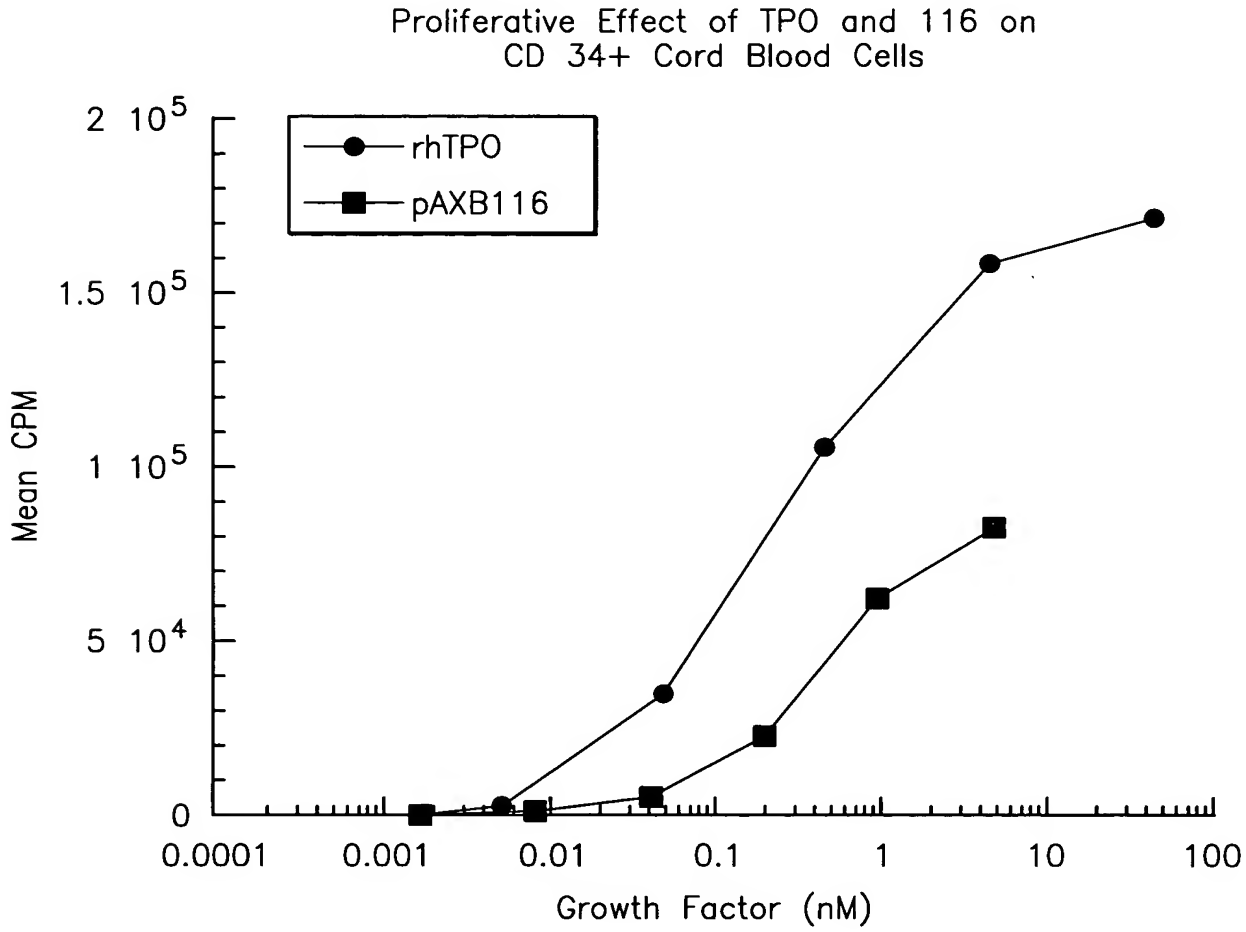


FIG. 31

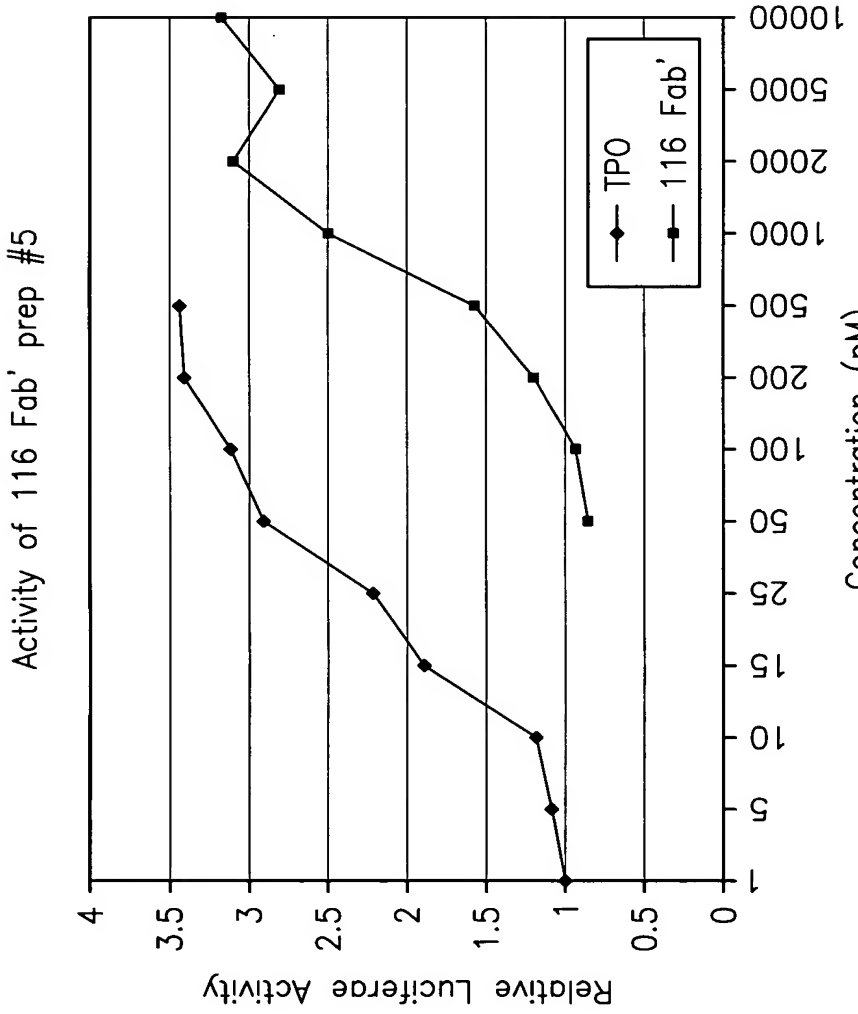

Activity of 116 Fab' prep #5
Relative Luciferae Activity
Concentration (pM)
TPO
116 Fab'
0
0.5
1
1.5
2
2.5
3
3.5
4
1
5
10
15
25
50
100
200
500
1000
2000
5000
10000

FIG. 32

Sample Sequences of Heavy Chain CDR2 clones

Gly-Ile-Phe-**xxx-xxx**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**xxx-xxx**-Gly (SEQ. ID NO. 126)

TT backbone **randomized** TPO peptide **randomized** TT backbone

Clone	Amino Acid Sequence	
HR2-14	Gly-Ile-Phe-**Ser-Pro**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Ala-Gly**-Gly	(SEQ. ID NO. 127)
HR2-20	Gly-Ile-Phe-**Pro-Gln**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Lys-His**-Gly	(SEQ. ID NO. 128)
HR2-23	Gly-Ile-Phe-**Pro-Asn**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Thr-Gly**-Gly	(SEQ. ID NO. 129)
HR2-28	Gly-Ile-Phe-**Lys-Gly**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Pro-Gly**-Gly	(SEQ. ID NO. 130)
HR2-43	Gly-Ile-Phe-**Pro-Pro**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Ala-Val**-Gly	(SEQ. ID NO. 131)
HR2-44	Gly-Ile-Phe-**Pro-Arg**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Lys-Leu**-Gly	(SEQ. ID NO. 132)
HR2-48	Gly-Ile-Phe-**Pro-Arg**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Lys-Leu**-Gly	(SEQ. ID NO. 133)
HR2-50	Gly-Ile-Phe-**Pro-Tyr**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Lys-Arg**-Gly	(SEQ. ID NO. 134)

FIG. 33

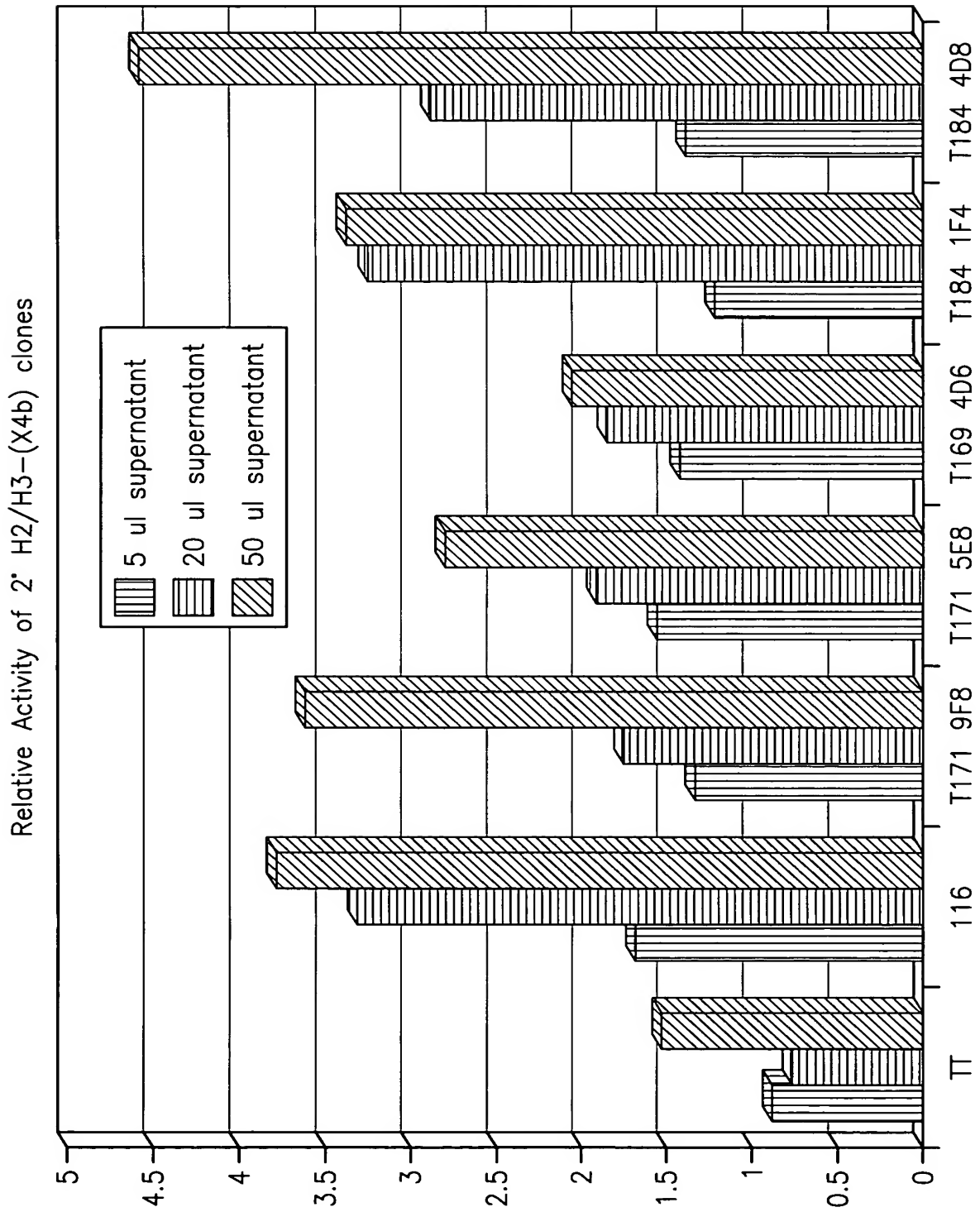

Relative Activity of 2° H2/H3-(X4b) clones
5 ul supernatant
20 ul supernatant
50 ul supernatant
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TT
116
T171 9F8
T171 5E8
T169 4D6
T184 1F4
T184 4D8

FIG. 34

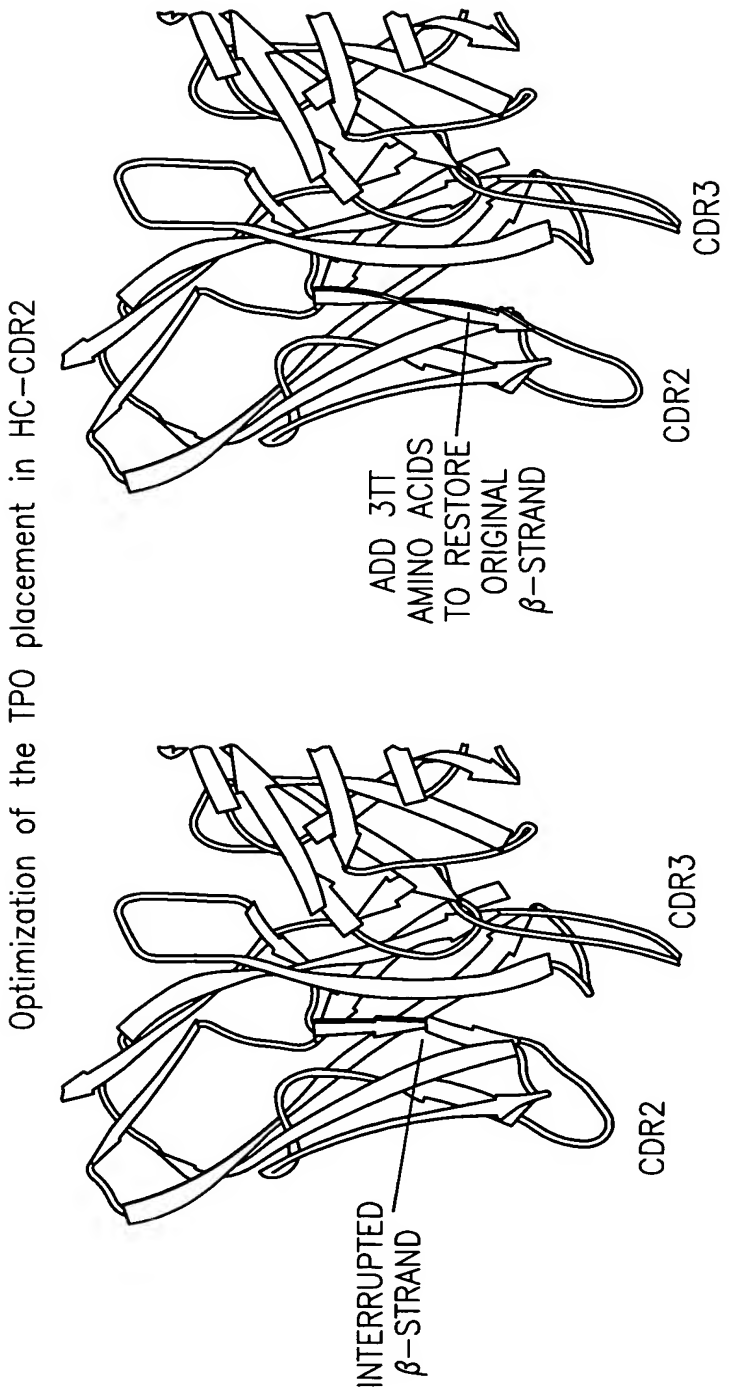
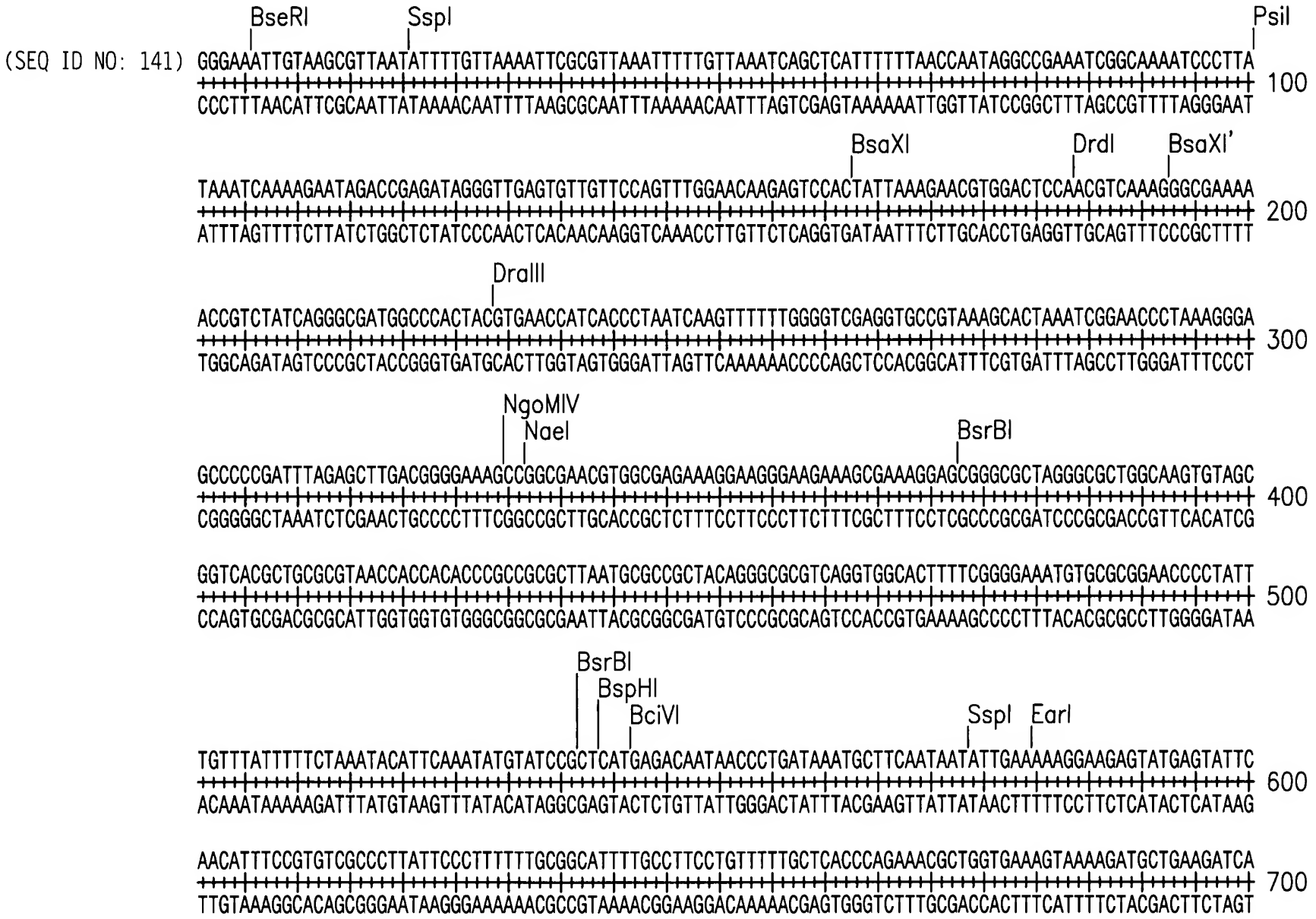

Optimization of the TPO placement in HC-CDR2
INTERRUPTED β-STRAND
CDR2
CDR3
ADD 3TT AMINO ACIDS TO RESTORE ORIGINAL β-STRAND
CDR2
CDR3

FIG. 35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PsiI
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BsaXI
DrdI
BsaXI'
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200
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DraIII
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300
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NgoMIV
NaeI
BsrBI
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400
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500
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BspHI
BciVI
SspI
EarI
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600
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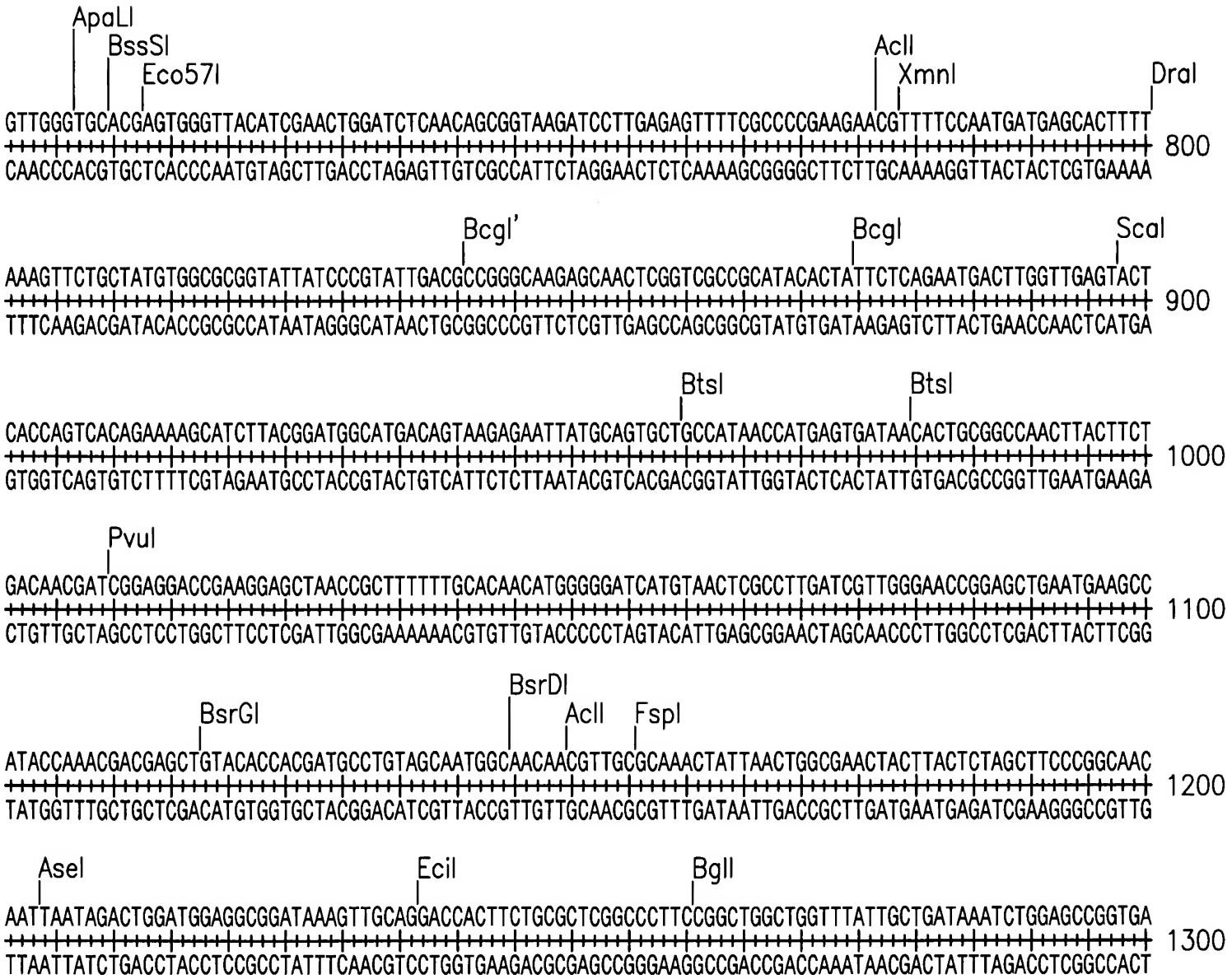
FIG. 36A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800
BcgI'
BcgI
ScaI
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900
BtsI
BtsI
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1000
PvuI
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1100
BsrDI
BsrGI
AclI
FspI
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AseI
EciI
BglI
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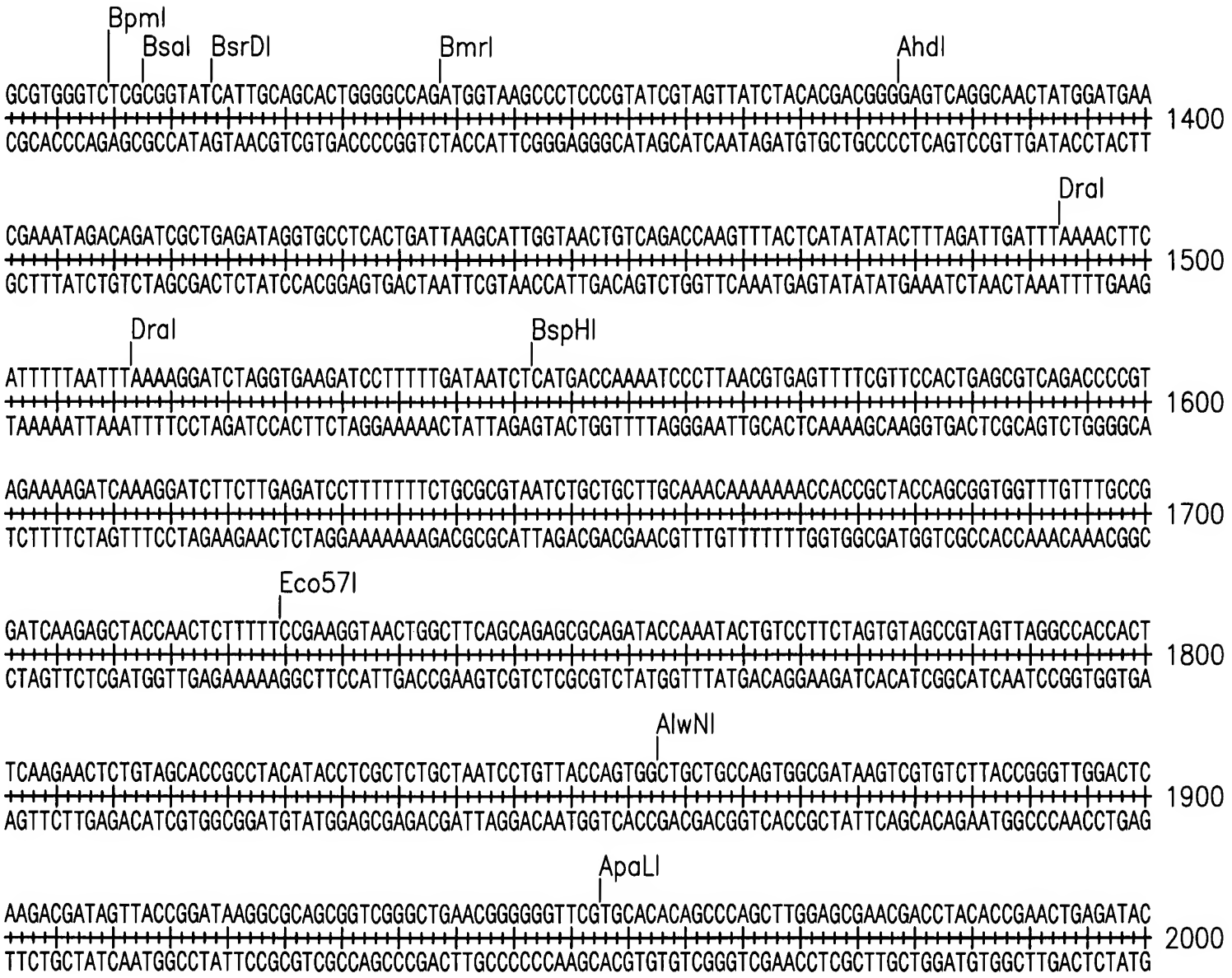
FIG. 36B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1400
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1500
DraI
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1700
Eco57I
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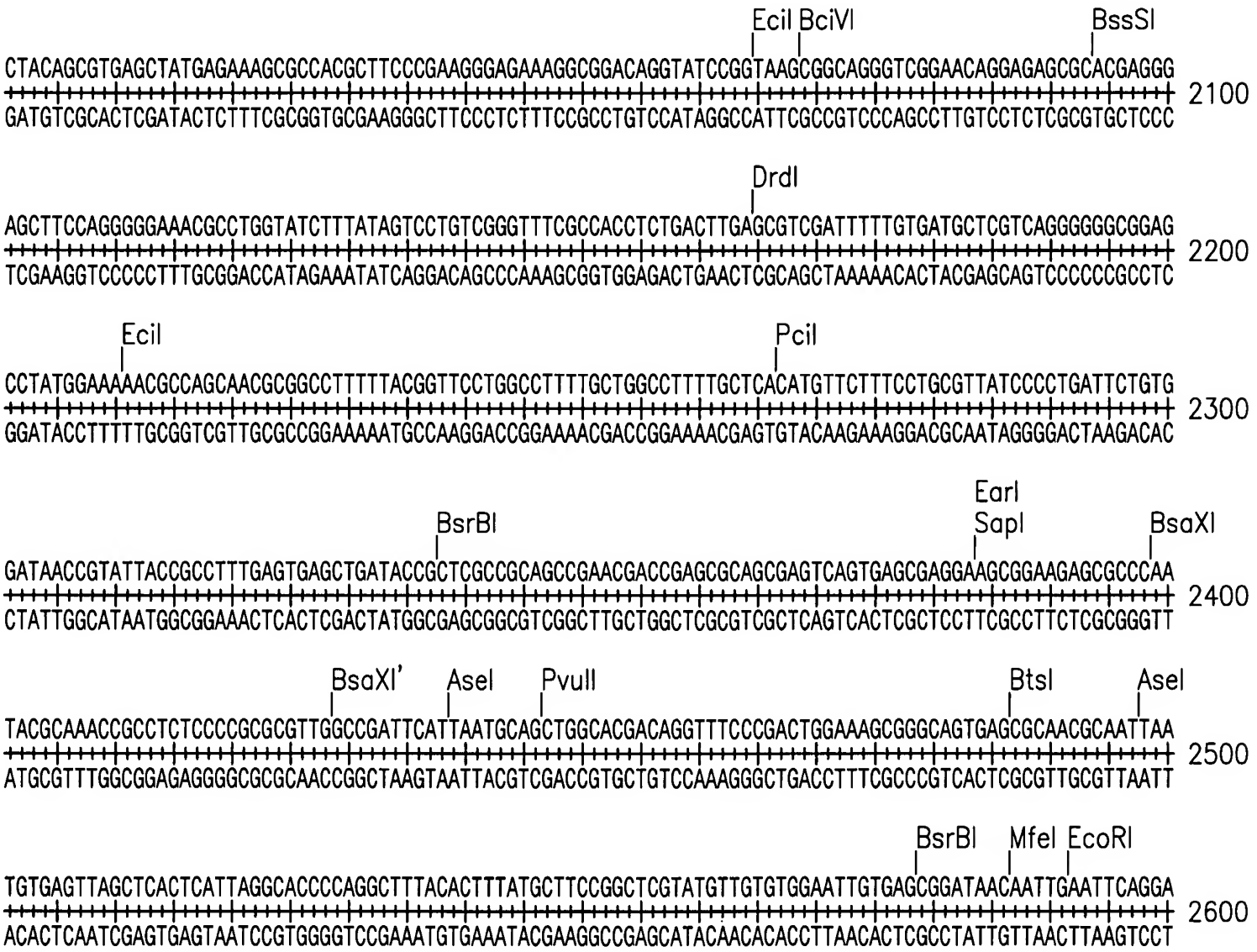
FIG. 36C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2100
DrdI
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2200
EciI
PciI
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EarI
SapI
BsaXI
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2400
BsaXI'
AseI
PvuII
BtsI
AseI
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MfeI
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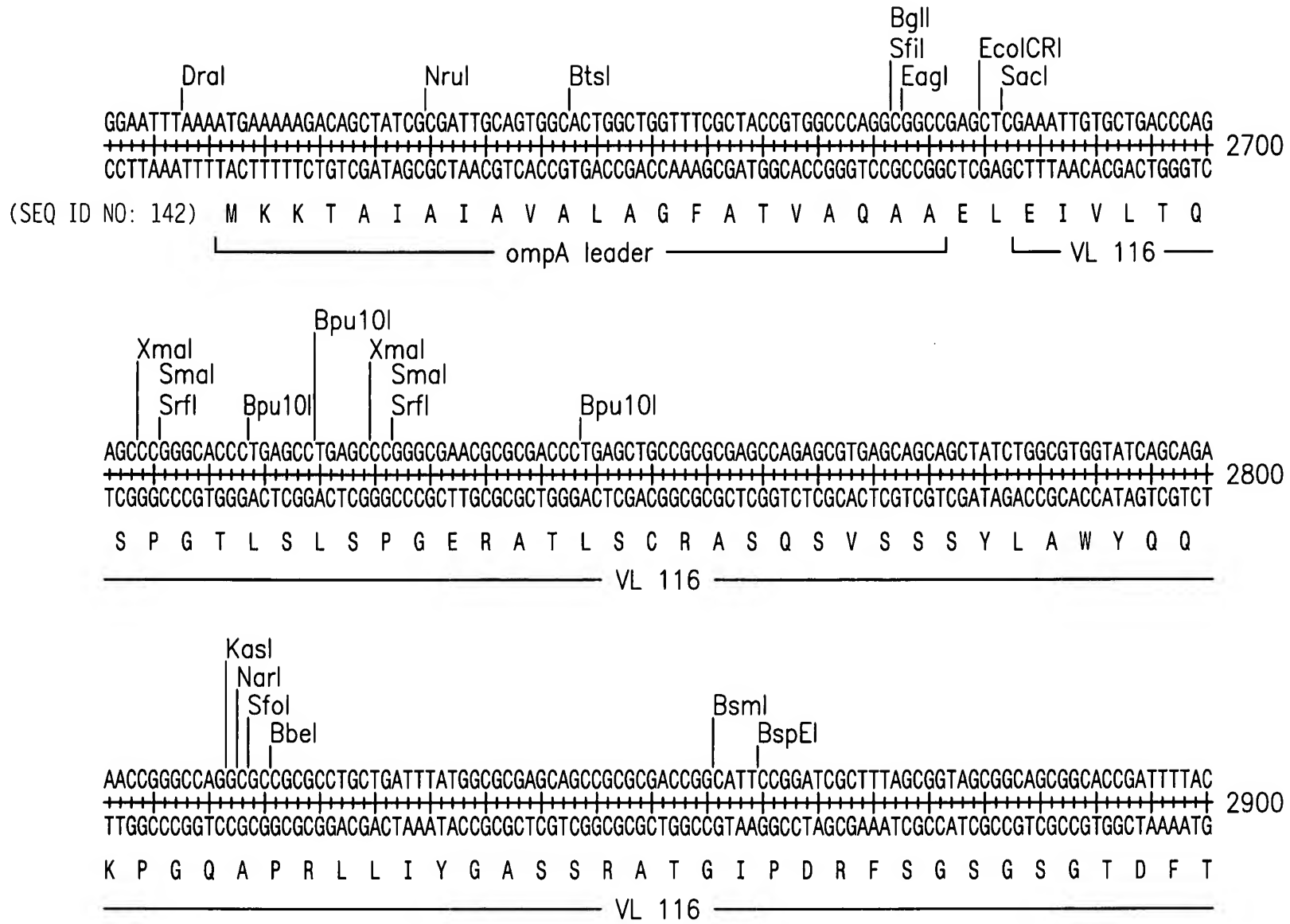
2600

FIG. 36D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2700
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VL 116
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XmaI
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SmaI
SrfI
Bpu10I
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VL 116
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NarI
SfoI
BbeI
BsmI
BspEI
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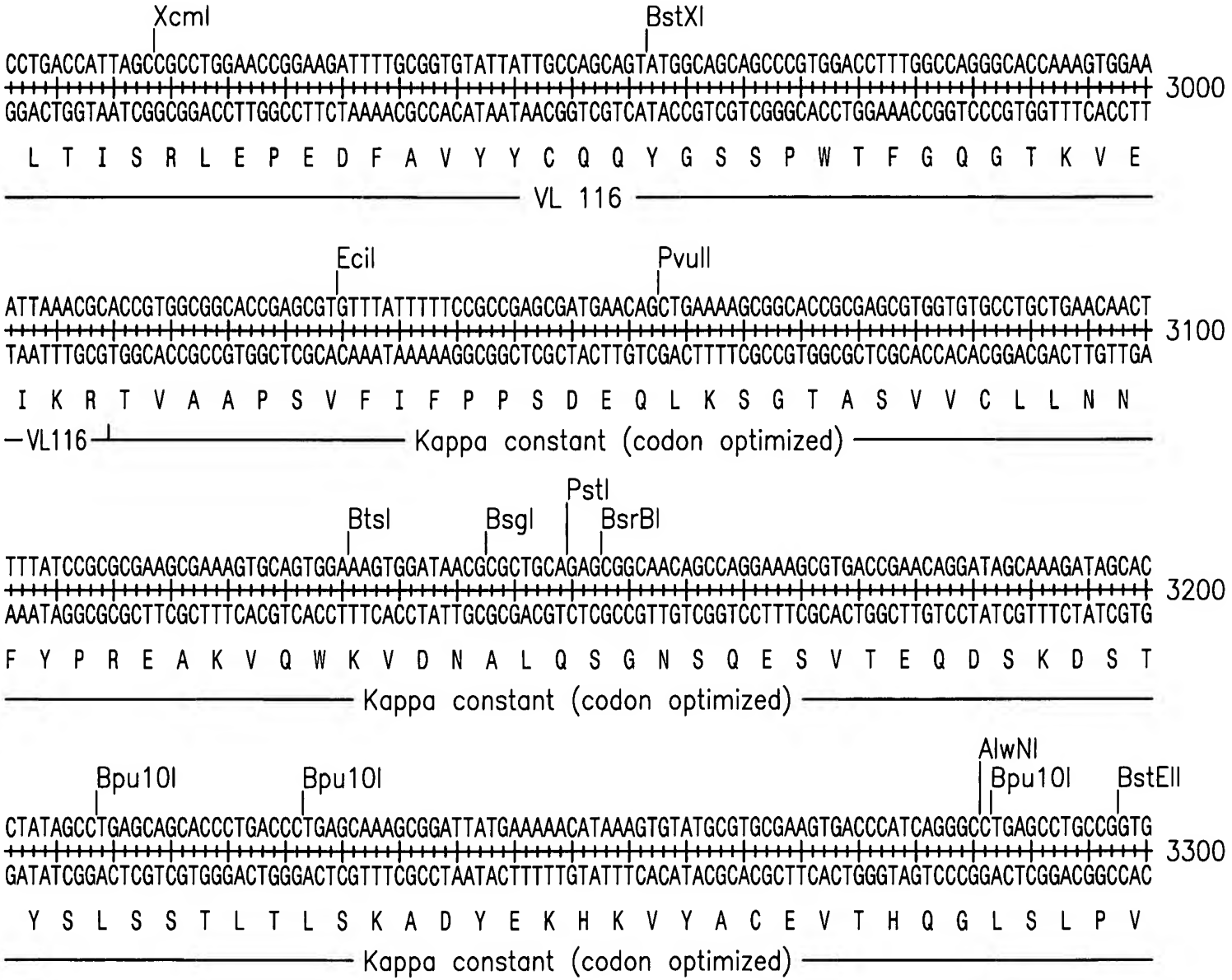
VL 116

FIG. 36E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3000
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VL 116
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PvuII
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I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L L N N
VL116
Kappa constant (codon optimized)
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BtsI
BsgI
BsrBI
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F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T
Kappa constant (codon optimized)
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Bpu10I
Bpu10I
Bpu10I
BstEII
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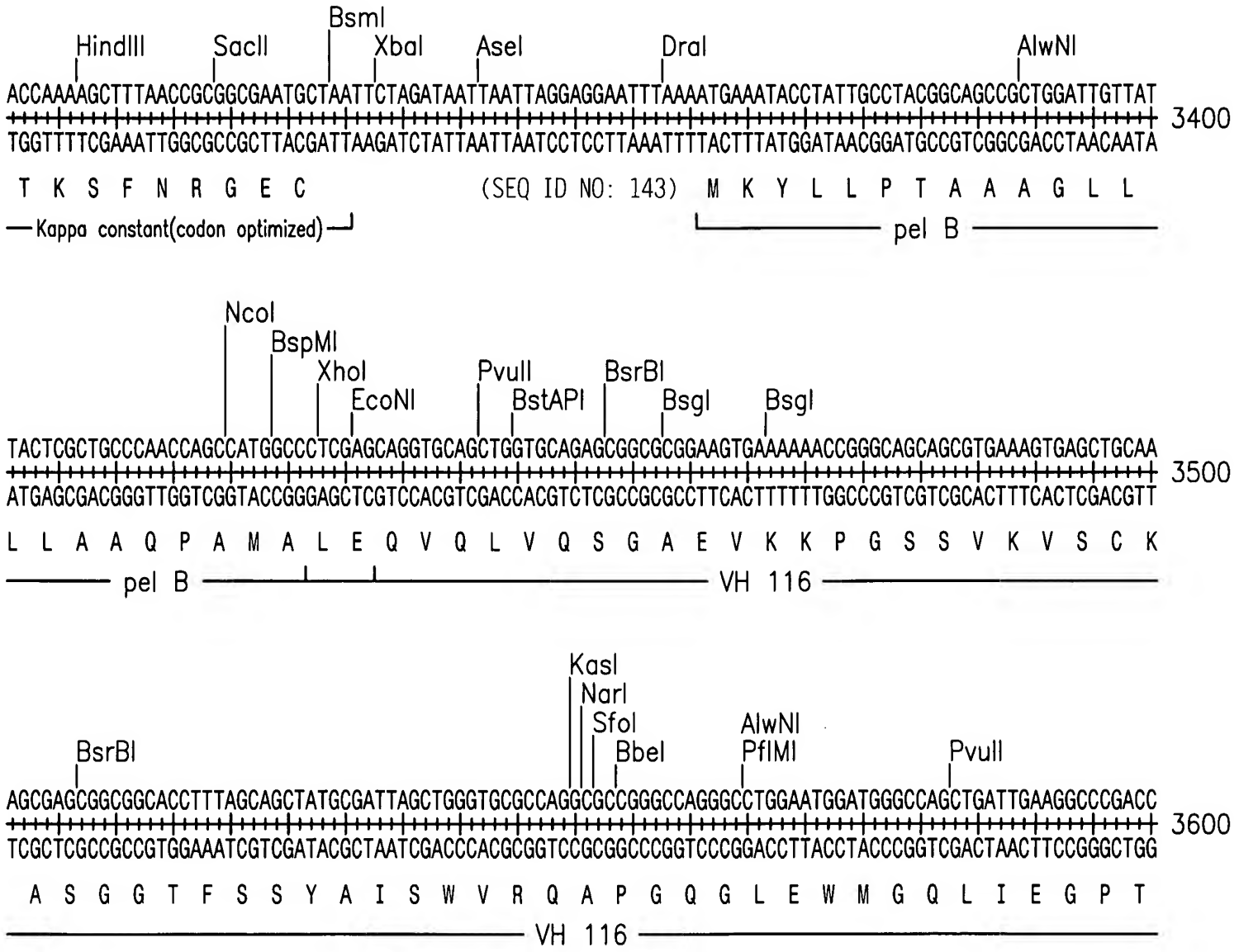
Kappa constant (codon optimized)

FIG. 36F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Kappa constant(codon optimized)
pel B
NcoI
BspMI
XhoI
EcoNI
PvuII
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BsrBI
BsgI
BsgI
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3500
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PflMI
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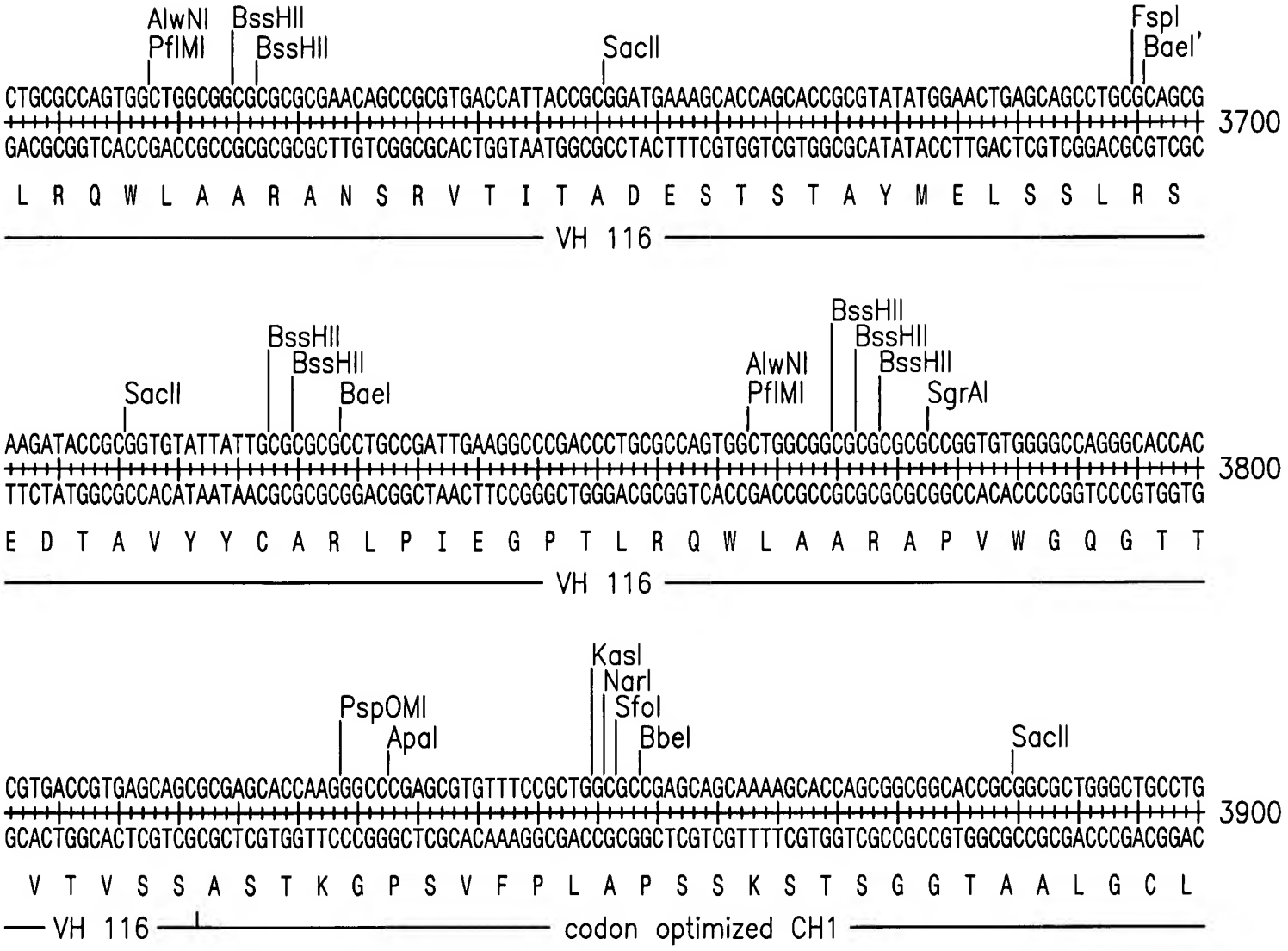
VH 116

FIG. 36G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3700
L R Q W L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S
VH 116
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VH 116
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BbeI
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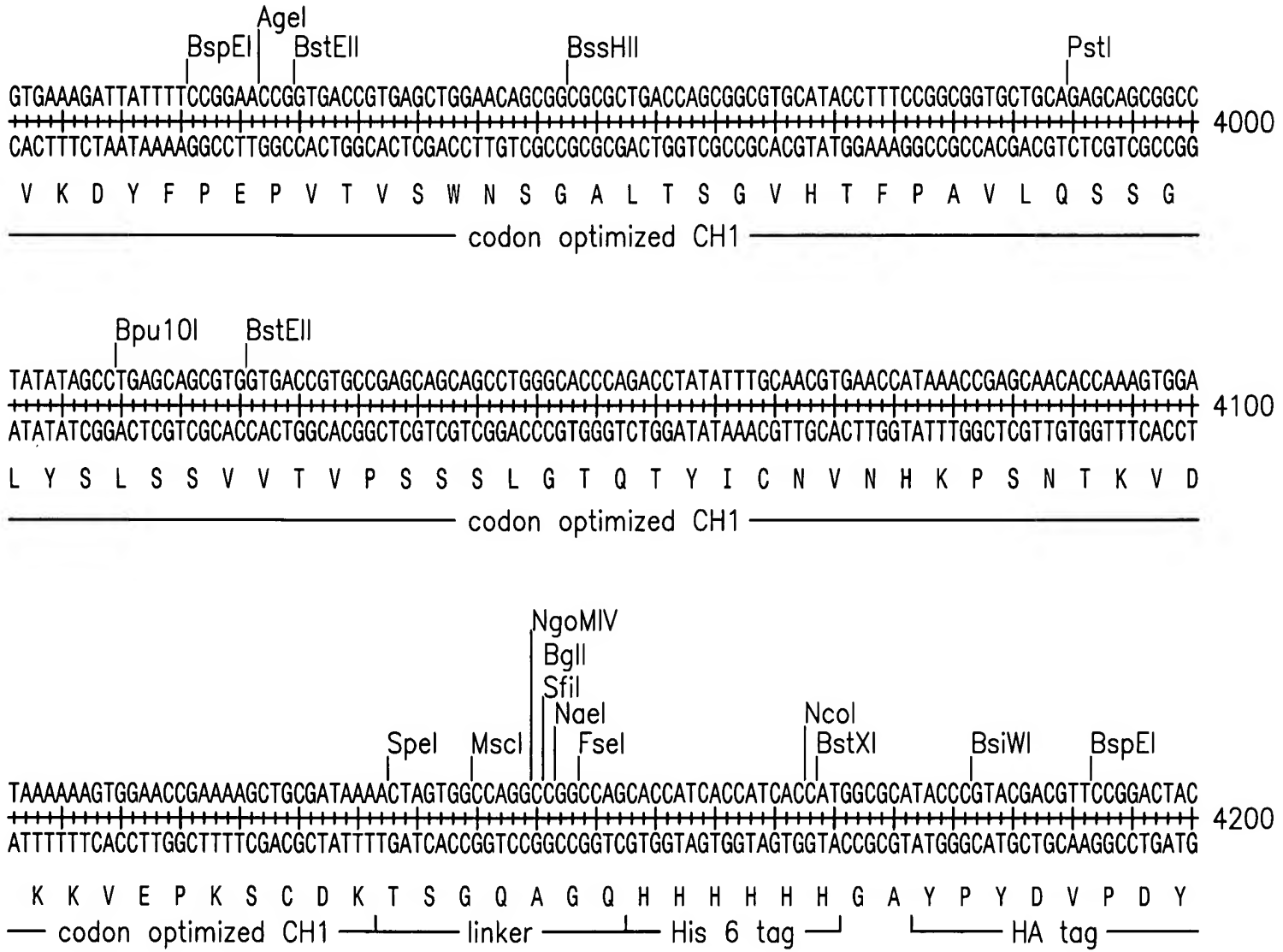
VH 116
codon optimized CH1

FIG. 36H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codon optimized CH1
Bpu10I
BstEII
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4100
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codon optimized CH1
NgoMIV
BglI
SfiI
NaeI
SpeI
MscI
FseI
NcoI
BstXI
BsiWI
BspEI
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ATTTTTTCACCTTGGCTTTTCGACGCTATTTTGATCACCGGTCCGGCCGGTCGTGGTAGTGGTAGTGGTACCGCGTATGGGCATGCTGCAAGGCCTGATG
4200
K K V E P K S C D K T S G Q A G Q H H H H H H G A Y P Y D V P D Y
codon optimized CH1
linker
His 6 tag
HA tag

FIG. 36I

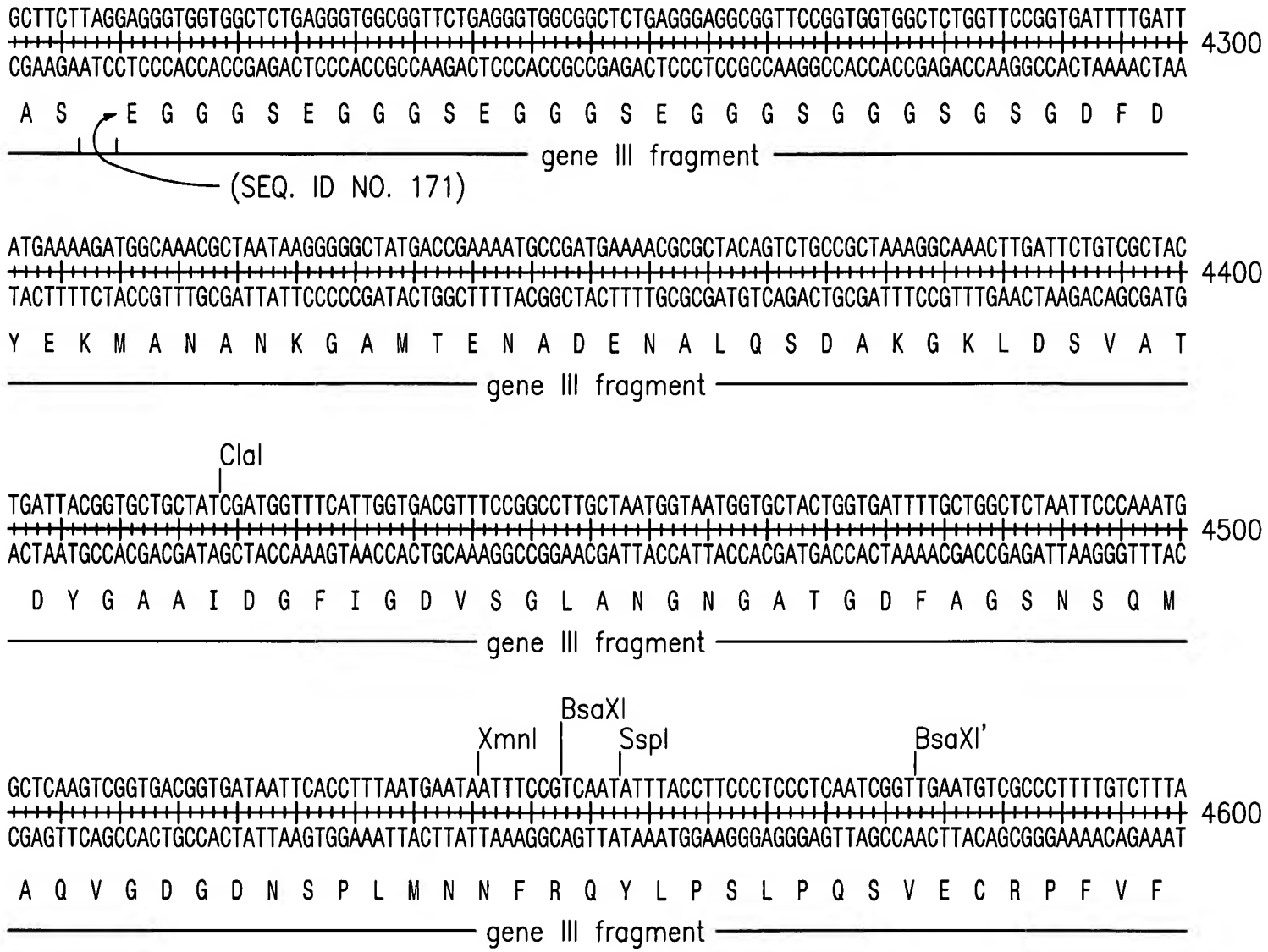

GCTTCTTAGGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGGCGGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATT
CGAAGAATCCTCCCACCACCGAGACTCCCACCGCCAAGACTCCCACCGCCGAGACTCCCTCCGCCAAGGCCACCACCGAGACCAAGGCCACTAAAACTAA
4300
A S E G G G S E G G G S E G G G S E G G G S G G G S G S G D F D
gene III fragment
(SEQ. ID NO. 171)
ATGAAAAGATGGCAAACGCTAATAAGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTCTGCCGCTAAAGGCAAACTTGATTCTGTCGCTAC
TACTTTTCTACCGTTTGCGATTATTCCCCCGATACTGGCTTTTACGGCTACTTTTGCGCGATGTCAGACTGCGATTTCCGTTTGAACTAAGACAGCGATG
4400
Y E K M A N A N K G A M T E N A D E N A L Q S D A K G K L D S V A T
gene III fragment
ClaI
TGATTACGGTGCTGCTATCGATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGTGATTTTGCTGGCTCTAATTCCCAAATG
ACTAATGCCACGACGATAGCTACCAAAGTAACCACTGCAAAGGCCGGAACGATTACCATTACCACGATGACCACTAAAACGACCGAGATTAAGGGTTTAC
4500
D Y G A A I D G F I G D V S G L A N G N G A T G D F A G S N S Q M
gene III fragment
BsaXI
XmnI
SspI
BsaXI'
GCTCAAGTCGGTGACGGTGATAATTCACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGAATGTCGCCCTTTTGTCTTTA
CGAGTTCAGCCACTGCCACTATTAAGTGGAAATTACTTATTAAAGGCAGTTATAAATGGAAGGGAGGGAGTTAGCCAACTTACAGCGGGAAAACAGAAAT
4600
A Q V G D G D N S P L M N N F R Q Y L P S L P Q S V E C R P F V F
gene III fragment

FIG. 36J

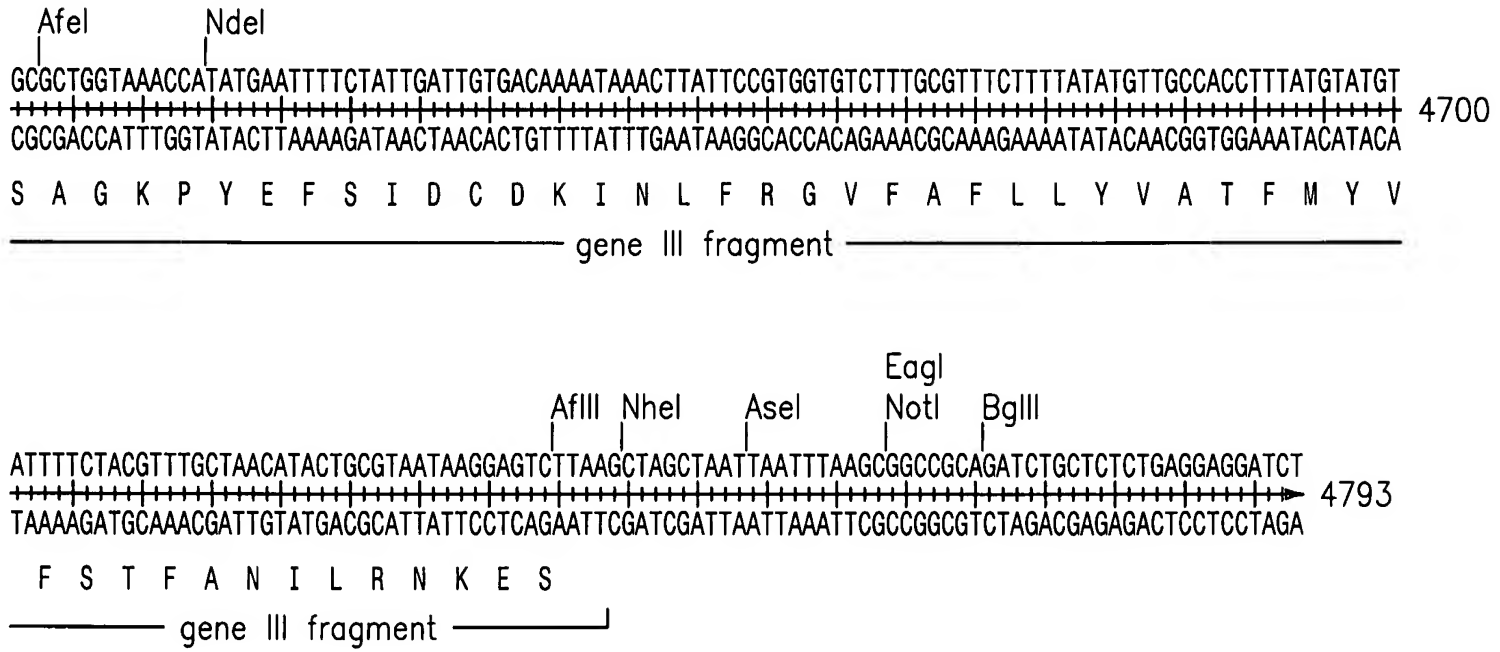

AfeI
NdeI
GCGCTGGTAAACCATATGAATTTTCTATTGATTGTGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCACCTTTATGTATGT
CGCGACCATTTGGTATACTTAAAAGATAACTAACACTGTTTTATTTGAATAAGGCACCACAGAAACGCAAAGAAAATATACAACGGTGGAAATACATACA
4700
S A G K P Y E F S I D C D K I N L F R G V F A F L L Y V A T F M Y V
gene III fragment
EagI
AflII
NheI
AseI
NotI
BglII
ATTTTCTACGTTTGCTAACATACTGCGTAATAAGGAGTCTTAAGCTAGCTAATTAATTTAAGCGGCCGCAGATCTGCTCTCTGAGGAGGATCT
TAAAAGATGCAAACGATTGTATGACGCATTATTCCTCAGAATTCGATCGATTAATTAAATTCGCCGGCGTCTAGACGAGAGACTCCTCCTAGA
4793
F S T F A N I L R N K E S
gene III fragment

FIG. 36K

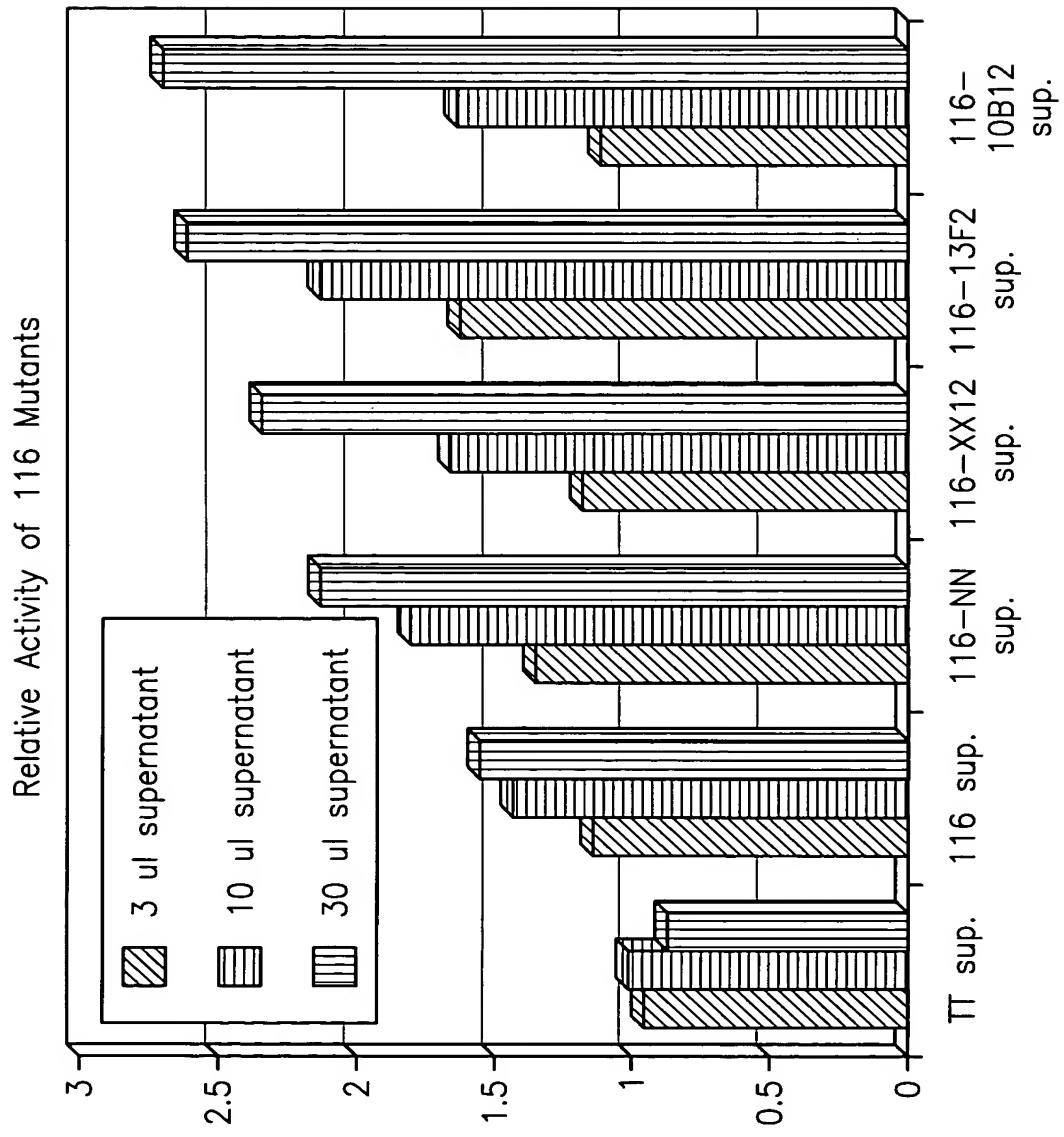
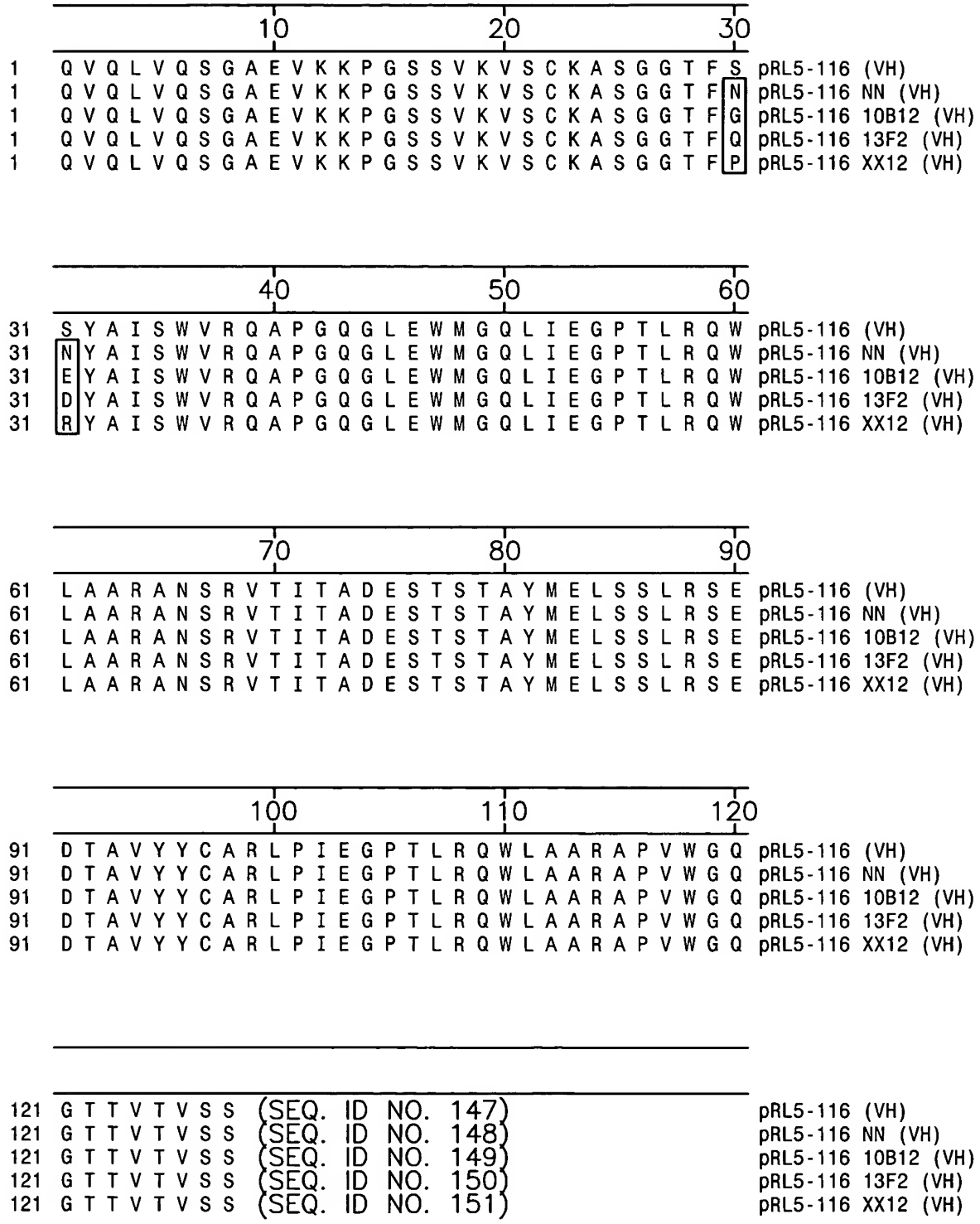

Relative Activity of 116 Mutants
3 ul supernatant
10 ul supernatant
30 ul supernatant
3
2.5
2
1.5
1
0.5
0
TT sup.
116 sup.
116–NN sup.
116–XX12 sup.
116–13F2 sup.
116–10B12 sup.

FIG. 37



116 Variants Alignment

FIG. 38